Data Evaluation Report on the acute toxicity of Prothioconazole on the Algae, *Anabaena flos-aquae*
PMRA Submission #:2004-0843 EPA MRID #: 46246103

Data Requirement:

PMRA DATA CODE	9.8.2
EPA DP Barcode	D303488
OECD Data Point	IIA 8.4.1
EPA MRID	46246103
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

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Date: 10/26/2005
10/26/05

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Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Kern, M.E., J.A. Roberts, and R.A. De Haan. 2004. Toxicity of JAU 6476 Technical to the Blue-green Alga *Anabaena flos-aquae*. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX078 (J6883801), and sponsored by Bayer CropScience, RTP, NC. Experimental start date December 9, 2002 and experimental termination date December 13, 2002. The final report issued February 23, 2004.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0 (negative and solvent controls), 0.02, 0.08, 0.27, 0.90, 3.0, and 10.0 ppm a.i.. The 0-hour measured concentrations were <0.5 (< LOQ, controls), 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i.; the 0-hour measured concentrations were used for toxicity estimates because the test substance declined to less than 70% of nominal for two test concentrations over the study period (nominal 0.08 and 0.27 ppm a.i.). The 96-hour cell density percent inhibitions were -6.2, -18.5, -17.3, 5.8, 35.8, and 91.4% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 9.1, 10.1, -21.3, 17.5, 41.5, and 83.2% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -1.6, -3.9, -3.6, 1.1, 10.0, and 60.2% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively. The NOAEC was 0.82 ppm a.i. for cell density and the NOAEC for growth rate and biomass was 2.97 ppm a.i.. Cell density and biomass (area under the growth curve) both had an EC_{50} of 3.5 ppm a.i..

The study is scientifically sound and satisfies the U.S. EPA Guideline §123-2 for an aquatic nonvascular plant study with *Anabaena flos-aquae*. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type: Static

Cell density:
NOAEC: 0.82 ppm a.i.
LOAEC: 2.97 ppm a.i.
EC_{05}: 1.1 ppm a.i. 95% C.I.: 0.57-2.1 ppm a.i.
EC_{50}/IC_{50}: 3.5 ppm a.i. 95% C.I.: 2.6-4.6 ppm a.i.
Slope: 3.29±0.608

Growth rate:
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
EC_{05}: 2.1 ppm a.i. 95% C.I.: 1.1-4.0 ppm a.i.
EC_{50}/IC_{50}: 7.4 ppm a.i. 95% C.I.: 6.3-8.7 ppm a.i.
Slope: 3.03±0.643

Plant biomass (area under the growth curve):
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
EC_{05}: 0.62 ppm a.i. 95% C.I.: 0.14-2.8 ppm a.i.
EC_{50}/IC_{50}: 3.5 ppm a.i. 95% C.I.: 2.0-6.0 ppm a.i.
Slope: 2.21±0.688

Endpoint(s) Affected: Cell density, growth rate, and biomass
Most sensitive endpoint: Cell density and 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 81-103% of the nominal concentrations and the 96 hour measured test concentrations were 58-96% of the nominal concentrations. In the two lowest test levels, the 96-hour measured concentration declined <70% of nominal. The other test levels exhibited stability over the course of the study.

(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: *Anabaena flos-aquae*

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: AF66

Source: Originally from University of Texas, Austin, Texas. Current in-house laboratory cultures.
Age of inoculum: 4 days old
Method of cultivation: 1X Algal Assay Procedure (AAP) medium

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 0.012, 0.12, 1.2, and 12 ppm a.i. with negative and solvent controls. The percent growth inhibitions were approximately 10, -3, 27, and 86% in the 0.012, 0.12, 1.2, and 12 ppm a.i. treatment groups, 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 toxicity observed)	Continuous 1X Algal Assay Procedure (AAP) 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*

Parameter	Details	Remarks *Criteria*
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.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	1X Algal Assay Procedure (AAP) medium 7.3-7.5 7.8-8.7 disodium EDTA $NaHCO_3$ N/A	*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:	 Freshwater medium Filter-sterilized (0.22 µm). 7.6 N/A 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), 0.02, 0.08, 0.27, 0.90, 3.0, and 10.0 ppm a.i. Day 0: <0.5 (< LOQ, controls), 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i.. Day 4: <0.5 (< LOQ, controls), 0.02, 0.05, 0.19, 0.86, 2.81, and 9.32 ppm 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/MS; 0 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	24.1-25.2°C Continuous 2.2 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 was 74-83X 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 compared to the pooled controls were -6.2, -18.5, -17.3, 5.8, 35.8, and 91.4% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions compared to the pooled controls were 9.1, 10.1, -21.3, 17.5, 41.5, and 83.2% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -1.6, -3.9, -3.6, 1.1, 10.0, and 60.2% compared to the pooled controls in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively.

Table 3: Effect of Prothioconazole on Algae (*Anabaena flos-aquae*)

Treatment day 0 measured and nominal concentrations [a] (ppm 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	29,000	741,000	--
Solvent control	10,000	21,000	834,000	--
0.02 (0.02)	10,000	29,000	837,000	-6.2
0.08 (0.08)	10,000	22,000	934,000	-18.5
0.22 (0.27)	10,000	43,000	924,000	-17.3
0.82 (0.90)	10,000	25,000	742,000	5.8
2.97 (3.0)	10,000	42,000	506,000*	35.8
9.12 (10.0)	10,000	21,000	68,000*	91.4
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. Negative percent inhibitions indicate promoted growth.
* Statistically significant from the pooled control (Dunnett's one tailed test).

Table 4: Effect of Prothioconazole on Algae *(Anabaena flos-aquae)*

Treatment day 0 measured and Concentrations [a] (ppm 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	--	0.04602	--	1901.0	--
0.02 (0.02)	10,000	0.04601	-1.6	1728.0	9.1
0.08 (0.08)	10,000	0.04708	-3.9	1708.4	10.1
0.22 (0.27)	10,000	0.04693	-3.6	2306.8	-21.3
0.82 (0.90)	10,000	0.04480	1.1	1568.4	17.5
2.97 (3.0)	10,000	0.04074*	10.0	1111.6	41.5
9.12 (10.0)	10,000	0.01803*	60.2	318.8*	83.2

Treatment day 0 measured and Concentrations [a] (ppm 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]
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. Negative percent inhibition indicates promoted growth.
* Statistically significant from the pooled control (Dunnett's one tailed test).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC or EC_{05} (ppm a.i.)	2.97	0.82	0.82
IC_{50} or EC_{50} (ppm a.i.)(and 95% C.I.)	3.55 (3.00-4.10)	9.12 (7.82-10.42)	3.71 (3.35-4.08)
IC_{25}/EC_{25} (ppm a.i.) (and 95% C.I.)	1.83 (1.35-2.32)	7.84 (0-2000.4)	2.44 (2.11-2.78)
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 58. 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 nonparametric analyses were conducted for growth rate, and the parametric analyses were conducted for density and biomass. 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: 0.82 ppm a.i.
LOAEC: 2.97 ppm a.i.
EC_{50}/IC_{50}: 3.71 ppm a.i. 95% C.I.: 3.35-4.08 ppm a.i.
Slope: N/A

Growth rates:
NOAEC: 0.82 ppm a.i.
LOAEC: 2.97 ppm a.i.

EC_{50}/IC_{50}: 9.12 ppm a.i. 95% C.I.: 7.82-10.42 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
EC_{50}/IC_{50}: 3.55 ppm a.i. 95% C.I.: 3.00-4.10 ppm 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: Cell density, area under the growth curve (biomass), and growth rate data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). The NOAEC and LOAEC were determined using ANOVA, followed by Bonferroni's t-test (non-monotonic response) or William's test (monotonic response). For all endpoints, the solvent control was compared to the test medium 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: 0.82 ppm a.i.
LOAEC: 2.97 ppm a.i.
EC_{05}: 1.1 ppm a.i. 95% C.I.: 0.57-2.1 ppm a.i.
EC_{50}/IC_{50}: 3.5 ppm a.i. 95% C.I.: 2.6-4.6 ppm a.i.
Slope: 3.29±0.608

Growth rate:
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
EC_{05}: 2.1 ppm a.i. 95% C.I.: 1.1-4.0 ppm a.i.
EC_{50}/IC_{50}: 7.4 ppm a.i. 95% C.I.: 6.3-8.7 ppm a.i.
Slope: 3.03±0.643

Plant biomass (area under the growth curve):
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
EC_{05}: 0.62 ppm a.i. 95% C.I.: 0.14-2.8 ppm a.i.
EC_{50}/IC_{50}: 3.5 ppm a.i. 95% C.I.: 2.0-6.0 ppm a.i.
Slope: 2.21±0.688

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

D. STUDY DEFICIENCIES:

There were no significant study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study author's; however, the reviewer's analysis revealed that cell density and biomass were equally sensitive endpoints with identical EC_{50} values. The reviewer calculated EC_{05} values for the purpose of endangered species risk assessment. The reviewer's estimates are associated with slope values and, so, 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. Cell density had the lowest NOAEC (0.82 ppm a.i.), while both cell density and biomass had the same EC_{50} value of 3.5 ppm a.i.

Cell density:
NOAEC: 0.82 ppm a.i.
LOAEC: 2.97 ppm a.i.
EC_{05}: 1.1 ppm a.i. 95% C.I.: 0.57-2.1 ppm a.i.
EC_{50}/IC_{50}: 3.5 ppm a.i. 95% C.I.: 2.6-4.6 ppm a.i.
Slope: 3.29±0.608

Growth rate:
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
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EC_{50}/IC_{50}: 7.4 ppm a.i. 95% C.I.: 6.3-8.7 ppm a.i.
Slope: 3.03±0.643

Plant biomass (area under the growth curve):
NOAEC: 2.97 ppm a.i.
LOAEC: 9.12 ppm a.i.
EC_{05}: 0.62 ppm a.i. 95% C.I.: 0.14-2.8 ppm a.i.
EC_{50}/IC_{50}: 3.5 ppm a.i. 95% C.I.: 2.0-6.0 ppm a.i.
Slope: 2.21±0.688

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

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6103cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	17333.865	2888.977	12.648
Within (Error)	17	3883.088	228.417	
Total	23	21216.953		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 6103cd 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	78.783	78.783		
2	0.02	83.667	83.667	-0.457	
3	0.08	93.367	93.367	-1.365	
4	0.22	92.367	92.367	-1.271	
5	0.82	74.167	74.167	0.432	
6	2.97	50.633	50.633	2.634	
7	9.12	6.767	6.767	6.739	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

cell density
File: 6103cd 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	6			
2	0.02	3	28.374	36.0	-4.883
3	0.08	3	28.374	36.0	-14.583
4	0.22	3	28.374	36.0	-13.583
5	0.82	3	28.374	36.0	4.617
6	2.97	3	28.374	36.0	28.150
7	9.12	3	28.374	36.0	72.017

cell density
File: 6103cd 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	6	78.783	78.783	85.393
2	0.02	3	83.667	83.667	85.393
3	0.08	3	93.367	93.367	85.393
4	0.22	3	92.367	92.367	85.393
5	0.82	3	74.167	74.167	74.167
6	2.97	3	50.633	50.633	50.633
7	9.12	3	6.767	6.767	6.767

cell density
File: 6103cd 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	85.393				
0.02	85.393	0.619		1.74	k= 1, v=17
0.08	85.393	0.619		1.82	k= 2, v=17
0.22	85.393	0.619		1.85	k= 3, v=17
0.82	74.167	0.432		1.87	k= 4, v=17
2.97	50.633	2.634	*	1.87	k= 5, v=17
9.12	6.767	6.739	*	1.88	k= 6, v=17

s = 15.113
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	1.1	0.57	2.1	0.14	0.52
EC10	1.4	0.80	2.5	0.12	0.57
EC25	2.2	1.4	3.3	0.089	0.65
EC50	3.5	2.6	4.6	0.060	0.75

Slope = 3.29 Std.Err. = 0.608

Goodness of fit: p = 0.53 based on DF= 4.0 17.

6103CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	78.8	84.0	-5.19	100.	0.00
0.0200	3.00	83.7	84.0	-0.307	100.	8.90e-12
0.0800	3.00	93.4	84.0	9.39	100.	3.64e-06
0.220	3.00	92.4	84.0	8.40	100.	0.00409
0.820	3.00	74.2	82.3	-8.15	98.0	1.97
2.97	3.00	50.6	49.3	1.30	58.7	41.3
9.12	3.00	6.77	7.02	-0.250	8.36	91.6

biomass
File: 6103b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	7745152.080	1290858.680	5.143
Within (Error)	17	4266725.040	250983.826	
Total	23	12011877.120		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

biomass
File: 6103b 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	1901.000	1901.000		
2	0.02	1728.000	1728.000	0.488	
3	0.08	1708.400	1708.400	0.544	
4	0.22	2306.800	2306.800	-1.146	
5	0.82	1568.400	1568.400	0.939	
6	2.97	1111.600	1111.600	2.228	
7	9.12	318.800	318.800	4.466	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

biomass
File: 6103b 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	6			
2	0.02	3	940.529	49.5	173.000
3	0.08	3	940.529	49.5	192.600
4	0.22	3	940.529	49.5	-405.800
5	0.82	3	940.529	49.5	332.600
6	2.97	3	940.529	49.5	789.400
7	9.12	3	940.529	49.5	1582.200

biomass
File: 6103b 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	6	1901.000	1901.000	1909.040

2	0.02	3	1728.000	1728.000	1909.040
3	0.08	3	1708.400	1708.400	1909.040
4	0.22	3	2306.800	2306.800	1909.040
5	0.82	3	1568.400	1568.400	1568.400
6	2.97	3	1111.600	1111.600	1111.600
7	9.12	3	318.800	318.800	318.800

biomass
File: 6103b 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	1909.040				
0.02	1909.040	0.023		1.74	k= 1, v=17
0.08	1909.040	0.023		1.82	k= 2, v=17
0.22	1909.040	0.023		1.85	k= 3, v=17
0.82	1568.400	0.939		1.87	k= 4, v=17
2.97	1111.600	2.228	*	1.87	k= 5, v=17
9.12	318.800	4.466	*	1.88	k= 6, v=17

s = 500.983
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	0.62	0.14	2.8	0.31	0.22
EC10	0.91	0.26	3.2	0.27	0.28
EC25	1.7	0.70	4.2	0.19	0.41
EC50	3.5	2.0	6.0	0.11	0.58

Slope = 2.21 Std.Err. = 0.688

Goodness of fit: p = 0.55 based on DF= 4.0 17.

6103B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	1.90e+03	1.89e+03	13.6	100.	0.00
0.0200	3.00	1.73e+03	1.89e+03	-159.	100.	3.77e-05
0.0800	3.00	1.71e+03	1.89e+03	-179.	100.	0.0150
0.220	3.00	2.31e+03	1.88e+03	427.	99.6	0.409
0.820	3.00	1.57e+03	1.73e+03	-161.	91.6	8.35
2.97	3.00	1.11e+03	1.05e+03	58.4	55.8	44.2
9.12	3.00	319.	332.	-13.2	17.6	82.4

growth rate
File: 6103g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F

Between	6	201401.303	33566.884	26.649
Within (Error)	17	21412.681	1259.569	
Total	23	222813.983		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

growth rate
File: 6103g 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	452.945	452.945		
2	0.02	460.113	460.113	-0.286	
3	0.08	470.760	470.760	-0.710	
4	0.22	469.287	469.287	-0.651	
5	0.82	448.010	448.010	0.197	
6	2.97	407.407	407.407	1.815	
7	9.12	180.320	180.320	10.863	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

growth rate
File: 6103g 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	6			
2	0.02	3	66.629	14.7	-7.168
3	0.08	3	66.629	14.7	-17.815
4	0.22	3	66.629	14.7	-16.342
5	0.82	3	66.629	14.7	4.935
6	2.97	3	66.629	14.7	45.538
7	9.12	3	66.629	14.7	272.625

growth rate
File: 6103g 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	6	452.945	452.945	461.210
2	0.02	3	460.113	460.113	461.210
3	0.08	3	470.760	470.760	461.210
4	0.22	3	469.287	469.287	461.210
5	0.82	3	448.010	448.010	448.010

6	2.97	3	407.407	407.407	407.407
7	9.12	3	180.320	180.320	180.320

growth rate
File: 6103g 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	461.210				
0.02	461.210	0.329		1.74	k= 1, v=17
0.08	461.210	0.329		1.82	k= 2, v=17
0.22	461.210	0.329		1.85	k= 3, v=17
0.82	448.010	0.197		1.87	k= 4, v=17
2.97	407.407	1.815		1.87	k= 5, v=17
9.12	180.320	10.863	*	1.88	k= 6, v=17

s = 35.490
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	2.1	1.1	4.0	0.13	0.53
EC10	2.8	1.7	4.7	0.11	0.60
EC25	4.4	3.2	6.1	0.067	0.73
EC50	7.4	6.3	8.7	0.034	0.85

Slope = 3.03 Std.Err. = 0.643

Goodness of fit: p = 0.97 based on DF= 4.0 17.

6103G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	453.	459.	-6.30	100.	0.00
0.0200	3.00	460.	459.	0.869	100.	3.47e-13
0.0800	3.00	471.	459.	11.5	100.	1.25e-07
0.220	3.00	469.	459.	10.0	100.	0.000183
0.820	3.00	448.	458.	-10.4	99.8	0.188
2.97	3.00	407.	407.	0.613	88.6	11.4
9.12	3.00	180.	180.	-0.0731	39.3	60.7

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: October 26, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Freshwater Algae (blue-green algae, *Anabaena flos-aquae*)

Kern, M.E., J.A. Roberts, and R.A. De Haan. 2004. Toxicity of JAU 6476 Technical to the Blue-green Alga *Anabaena flos-aquae*. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX078 (J6883801), and sponsored by Bayer CropScience, RTP, NC. Bayer Report No. 200497. Experimental start date December 9, 2002 and experimental termination date December 13, 2002. The final report issued February 23, 2004.

PMRA DATA CODE: 9.8.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.4.1
EPA MRID: 46246103
EPA Guideline: 123-2 (OPPTS 850.5400)

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to Prothioconazole (JAU 6476 Technical; purity 98.2%) under static conditions at nominal concentrations of 0 (negative and solvent controls), 0.02, 0.08, 0.27, 0.90, 3.0, and 10.0 mg a.i./L. The study was conducted following U.S. EPA Guideline 123-2 and in compliance with U.S. EPA (40 CFR, Part 160). The 0-hour measured concentrations were <0.5 (< LOQ, controls), 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 mg a.i./L; while the day-4 measured concentrations were <0.5 (< LOQ, controls), 0.02, 0.05, 0.19, 0.86, 2.81 and 9.32 mg a.i./L. According to US EPA policy, the US EPA used the 0-hour measured concentrations for toxicity estimates because the test substance declined to less than 70% of nominal for two test concentrations over the study period (nominal 0.08 and 0.27 mg a.i./L). The EAD reviewer finds the values chosen acceptable as differences in results expressed in terms of measured day 0, mean measured or measured day 4 concentrations are very small and are unlikely to affect the outcome of risk assessments. The 96-hour cell density percent inhibitions were -6.2, -18.5, -17.3, 5.8, 35.8, and 91.4% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 mg a.i./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 9.1, 10.1, -21.3, 17.5, 41.5, and 83.2% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 mg a.i./L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -1.6, -3.9, -3.6, 1.1, 10.0, and 60.2% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 mg a.i./L treatment groups, respectively. The NOEC was 0.82 mg a.i./L for cell density and the NOEC for growth rate and biomass was 2.97 mg

a.i./L. Cell density and biomass (area under the growth curve) both had an EC_{50}/IC_{50} of 3.5 mg a.i./L, while the EC_{50}/IC_{50} was 7.4 mg a.i./L for growth rate.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type: Static

Cell density:
NOEC: 0.82 mg a.i./L
LOEC: 2.97 mg a.i./L
EC_{05}: 1.1 mg a.i./L 95% C.I.: 0.57-2.1 mg a.i./L
EC_{50}/IC_{50}: 3.5 mg a.i./L 95% C.I.: 2.6-4.6 mg a.i./L
Slope: 3.29±0.608

Growth rate:
NOEC: 2.97 mg a.i./L
LOEC: 9.12 mg a.i./L
EC_{05}: 2.1 mg a.i./L 95% C.I.: 1.1-4.0 mg a.i./L
EC_{50}/IC_{50}: 7.4 mg a.i./L 95% C.I.: 6.3-8.7 mg a.i./L
Slope: 3.03±0.643

Plant biomass (area under the growth curve):
NOEC: 2.97 mg a.i./L
LOEC: 9.12 mg a.i./L
EC_{05}: 0.62 mg a.i./L 95% C.I.: 0.14-2.8 mg a.i./L
EC_{50}/IC_{50}: 3.5 mg a.i./L 95% C.I.: 2.0-6.0 mg a.i./L
Slope: 2.21±0.688

Endpoint(s) Affected: Cell density, growth rate, and biomass
Most sensitive endpoint: Cell density and 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 EAD reviewer verified the biomass and growth rate at 96 hours, based on the cell density and obtained identical results as the study authors.

3. After a review of the data and of the results, the EAD reviewer did not feel that redoing the statistical analyses to determine the NOECs was warranted. The EAD reviewer agrees with the NOECs determined by the study authors and the EPA reviewer.

4. The EAD reviewer verified the 96-hour EC_{50}/IC_{50} for cell density, cumulative biomass as well as growth rate using linear interpolation (Icp, US EPA, 1993). The reviewer estimated the EC_{50}/IC_{50} using both the mean measured concentrations as well as the day 4 measured concentrations. Mean measured concentrations were <0.5 (< LOQ, controls), 0.02, 0.06, 0.20, 0.84, 2.89 and 9.22 mg a.i./L. Irrespective of which concentrations were used, results obtained were within the confidence intervals of or were very close to the results reported by the EPA reviewer. The slight difference in values is unlikely to result in significant differences in risk assessments. The values reported by the EPA reviewer are therefore acceptable to EAD. As recovery after 4 days was above 90% in the treatment levels corresponding to the NOECs, there is little difference between using the day-0 measured values, the day-4 measured values or the mean measured values. The values reported by the EPA reviewer are therefore acceptable.

Study Acceptability: This study is scientifically sound and satisfies the data requirements for an aquatic nonvascular plant study with *Anabaena flos-aquae*. This study is classified as ACCEPTABLE.