Data Evaluation Report on the acute toxicity of Prothioconazole formulation on the Algae, *Pseudokirchneriella subcapitata*

PMRA Submission #: 2004-0843 EPA MRID #: 46246106

Data Requirement:

PMRA DATA CODE	9.8.6
EPA DP Barcode	D303495
OECD Data Point	IIIA 10.2.1.11
EPA MRID	46246106
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: JAU 6476 480 SC **Purity:** 43.0%

Common name: Prothioconazole formulation

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.: 178928-70-6

Synonyms: JAU6476

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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 Lam, C.V. 2004. Toxicity of JAU 6476 480 SC to the Green Alga *Pseudokirchneriella subcapitata* (a.k.a. *Selenastrum capricornutum*). Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX075 (J6883501), and sponsored by Bayer CropScience, RTP, NC. Experimental start date September 9, 2002 and experimental termination date September 13, 2002. The final report issued January 23, 2004.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to Prothioconazole formulation (JAU 6476 480 SC) under static conditions at nominal concentrations of 0 (negative and formulation blank controls), 0.063, 0.125, 0.25, 0.5, and 1.0 and 2.0 ppm a.i.. The 0-hour measured concentrations were <0.021 (< LOQ, controls), 0.053, 0.111, 0.24, 0.48, 0.92, and 1.97 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 the two lowest test concentrations over the study period. The 96-hour cell density percent inhibitions were -3, -3, 17, 1, 37, and 94% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 0, 2, 16, 10, 52, and 95% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -1, -1, 3, 0, 8, and 52% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 ppm a.i. treatment groups, respectively. The NOAEC for all endpoints was 0.48 ppm a.i.. Biomass (area under the growth curve) was the most sensitive endpoint tested with an EC_{50} of 0.92 ppm a.i..

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

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type: Static

Cell density:
NOAEC: 0.48 ppm a.i.
EC_{05}: 0.56 ppm a.i. 95% C.I.: 0.43-0.72 ppm a.i.
EC_{50}/IC_{50}: 1.1 ppm a.i. 95% C.I.: 0.94-1.2 ppm a.i.
Slope: 5.88±0.718

Growth rates:
NOAEC: 0.48 ppm a.i.
EC_{05}: 0.82 ppm a.i. 95% C.I.: 0.71-0.94 ppm a.i.
EC_{50}/IC_{50}: 1.9 ppm a.i. 95% C.I.: 1.9-2.0 ppm a.i.
Slope: 4.39±0.340

Plant biomass (area under the growth curve):
NOAEC: 0.48 ppm a.i.
EC_{05}: 0.43 ppm a.i. 95% C.I.: 0.34-0.53 ppm a.i.
EC_{50}/IC_{50}: 0.92 ppm a.i. 95% C.I.: 0.83-1.0 ppm a.i.
Slope: 4.94±0.459

Endpoint(s) Affected: Cell density, growth rates, 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 123-3, 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 formulation (JAU 6476 480 SC)

Description: White, milky liquid.

Lot No./Batch No. : 003-0115

Purity: 43.0%

Stability of Compound Under Test Conditions: The 0 hour measured test concentrations were 84-98% of the nominal concentrations and the 96 hour measured test concentrations were 32-89% of the nominal concentrations.

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

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

2. Test organism:

Name: *Pseudokirchneriella subcapitata*

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: SC-202
Source: Originally from University of Texas, Austin, Texas. Current in-house laboratory cultures.
Age of inoculum: 3 days old
Method of cultivation: Algal Assay Procedure (AAP) medium

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The preliminary studies included initial rangefinder and definitive studies. These studies were not considered due to growth variability and a test solution mixing error. An additional study was conducted at 0.125, 0.25, 0.50, 1.0, and 2.0 ppm a.i. with negative and formulation blank controls. The percent growth inhibition ranged from 28 to 94% and a NOAEC was not observed.

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 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):**	Algal Assay Procedure (AAP) medium 7.3-7.5 7.7-9.4 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). 7.5 N/A None Not reported Not reported Not reported Not detected 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 (formulation blank) 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 formulation blank controls), 0.063, 0.125, 0.25, 0.5, and 1.0 and 2.0 ppm a.i. Day 0: <0.021 (< LOQ, controls), 0.053, 0.111, 0.24, 0.48, 0.92, and 1.97 ppm a.i.. Day 4: <0.021 (< LOQ, controls), 0.02, 0.066, 0.20, 0.42, 0.83, and 1.78 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)	N/A	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification	HPLC/MS; 0 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	24.8-25.3°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 haemocytometer.	*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 formulation blank control cell densities at test termination was 52-53X greater than the dilution water control and formulation blank 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 -3, -3, 17, 1, 37, and 94% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 0, 2, 16, 10, 52, and 95% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -1, -1, 3, 0, 8, and 52% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 ppm a.i. treatment groups, respectively.

Table 3: Effect of Prothioconazole formulation on Algae (*Pseudokirchneriella subcapitata*)

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	49,000	2,635,000	--
Formulation blank control	10,000	47,000	2,641,000	--
0.053 (0.063)	10,000	42,000	2,613,000	-3
0.111 (0.125)	10,000	46,000	2,608,000	-3
0.24 (0.25)	10,000	41,000	2,101,000	17
0.48 (0.5)	10,000	39,000	2,511,000	1
0.92 (1.0)	10,000	29,000	1,597,000*	37
1.97 (2.0)	10,000	18,000	146,000*	94
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 formulation on Algae *(Pseudokirchneriella subcapitata)*

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	--	--	--	--
Formulation blank control	10,000	--	--	--	--
Pooled control	--	0.05758	--	6897.8	--
0.053 (0.063)	10,000	0.05790	-1	6900.8	0
0.111 (0.125)	10,000	0.05788	-1	6788.4	2
0.24 (0.25)	10,000	0.05568	3	5767.2	16
0.48 (0.5)	10,000	0.05748	0	6235.2	10
0.92 (1.0)	10,000	0.05283*	8	3310.4*	52
1.97 (2.0)	10,000	0.02785*	52	342.8*	95

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.)	0.48	0.48	0.48
EC_{50} (ppm a.i.)	0.90	1.93	1.04
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	0.88-0.92	1.91-1.96	1.01-1.06
IC_{25}/EC_{25} (ppm a.i.) (and 95% C.I.)	0.67 (0.64-0.70)	1.38 (1.31-1.45)	0.81 (0.78-0.84)
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 51. The 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 non-linear regression model. The analyses were conducted using the computer program SAS version8. All statistical calculations were performed using the day 0 measured concentrations.

Cell density:
NOAEC: 0.48 ppm a.i.
LOAEC: 0.92 ppm a.i.
EC_{50}/IC_{50}: 1.04 ppm a.i. 95% C.I.: 1.01-1.06 ppm a.i.
Slope: N/A

Growth rates:

NOAEC: 0.48 ppm a.i.
LOAEC: 0.92 ppm a.i.
EC_{50}/IC_{50}: 1.93 ppm a.i. 95% C.I.: 1.91-1.96 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 0.48 ppm a.i.
LOAEC: 0.92 ppm a.i.
EC_{50}/IC_{50}: 0.90 ppm a.i. 95% C.I.: 0.88-0.92 ppm a.i.
Slope: N/A

Endpoint(s) Affected: Cell density, growth rates, and 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. 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: 0.48 ppm a.i.
EC_{05}: 0.56 ppm a.i. 95% C.I.: 0.43-0.72 ppm a.i.
EC_{50}/IC_{50}: 1.1 ppm a.i. 95% C.I.: 0.94-1.2 ppm a.i.
Slope: 5.88±0.718

Growth rates:
NOAEC: 0.48 ppm a.i.
EC_{05}: 0.82 ppm a.i. 95% C.I.: 0.71-0.94 ppm a.i.
EC_{50}/IC_{50}: 1.9 ppm a.i. 95% C.I.: 1.9-2.0 ppm a.i.
Slope: 4.39±0.340

Plant biomass (area under the growth curve):
NOAEC: 0.48 ppm a.i.
EC_{05}: 0.43 ppm a.i. 95% C.I.: 0.34-0.53 ppm a.i.
EC_{50}/IC_{50}: 0.92 ppm a.i. 95% C.I.: 0.83-1.0 ppm a.i.
Slope: 4.94±0.459

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

D. STUDY DEFICIENCIES:

There were no significant study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the NOAEC and EC_{50} values for all endpoints were similar to the study authors'. Because the reviewer's estimates were associated with slope values, 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 with an EC_{50} of 0.92 ppm a.i..

Cell density:
NOAEC: 0.48 ppm a.i.
EC_{05}: 0.56 ppm a.i. 95% C.I.: 0.43-0.72 ppm a.i.
EC_{50}/IC_{50}: 1.1 ppm a.i. 95% C.I.: 0.94-1.2 ppm a.i.
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EC_{50}/IC_{50}: 0.92 ppm a.i. 95% C.I.: 0.83-1.0 ppm a.i.
Slope: 4.94±0.459

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

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6106cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	154701.221	25783.537	24.157
Within (Error)	17	18144.755	1067.339	
Total	23	172845.976		

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

cell density
File: 6106cd 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	254.550	254.550		
2	0.053	261.333	261.333	-0.294	
3	0.111	260.767	260.767	-0.269	
4	0.24	210.133	210.133	1.923	
5	0.48	251.067	251.067	0.151	
6	0.92	159.733	159.733	4.104	*
7	1.97	14.567	14.567	10.388	*

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

cell density
File: 6106cd 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	0.053	3	61.334	24.1	-6.783
3	0.111	3	61.334	24.1	-6.217
4	0.24	3	61.334	24.1	44.417
5	0.48	3	61.334	24.1	3.483
6	0.92	3	61.334	24.1	94.817
7	1.97	3	61.334	24.1	239.983

cell density
File: 6106cd 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	254.550	254.550	257.800
2	0.053	3	261.333	261.333	257.800
3	0.111	3	260.767	260.767	257.800
4	0.24	3	210.133	210.133	230.600
5	0.48	3	251.067	251.067	230.600
6	0.92	3	159.733	159.733	159.733
7	1.97	3	14.567	14.567	14.567

cell density
File: 6106cd 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	257.800				
0.053	257.800	0.141		1.74	k= 1, v=17
0.111	257.800	0.141		1.82	k= 2, v=17
0.24	230.600	1.037		1.85	k= 3, v=17
0.48	230.600	1.037		1.87	k= 4, v=17
0.92	159.733	4.104	*	1.87	k= 5, v=17
1.97	14.567	10.388	*	1.88	k= 6, v=17

s = 32.670
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	0.56	0.43	0.72	0.053	0.78
EC10	0.64	0.52	0.80	0.046	0.80
EC25	0.82	0.69	0.97	0.035	0.84
EC50	1.1	0.94	1.2	0.026	0.88

Slope = 5.88 Std.Err. = 0.718

Goodness of fit: p = 0.30 based on DF= 4.0 17.

6106CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	255.	249.	5.10	100.	0.00
0.0530	3.00	261.	249.	11.9	100.	9.34e-13
0.111	3.00	261.	249.	11.3	100.	3.93e-07
0.240	3.00	210.	249.	-39.3	100.	0.00717
0.480	3.00	251.	244.	6.87	97.9	2.11
0.920	3.00	160.	161.	-1.08	64.5	35.5
1.97	3.00	14.6	14.4	0.141	5.78	94.2

biomass
File: 6106b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	118321547.219	19720257.870	48.884
Within (Error)	17	6857955.119	403409.125	
Total	23	125179502.338		

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

biomass
File: 6106b 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	6897.800	6897.800		
2	0.053	6900.800	6900.800	-0.007	
3	0.111	6788.400	6788.400	0.244	
4	0.24	5767.200	5767.200	2.517	
5	0.48	6235.200	6235.200	1.475	
6	0.92	3310.400	3310.400	7.988	*
7	1.97	342.800	342.800	14.595	*

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

biomass
File: 6106b 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	0.053	3	1192.401	17.3	-3.000
3	0.111	3	1192.401	17.3	109.400
4	0.24	3	1192.401	17.3	1130.600
5	0.48	3	1192.401	17.3	662.600
6	0.92	3	1192.401	17.3	3587.400
7	1.97	3	1192.401	17.3	6555.000

biomass
File: 6106b 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	6897.800	6897.800	6898.800
2	0.053	3	6900.800	6900.800	6898.800
3	0.111	3	6788.400	6788.400	6788.400
4	0.24	3	5767.200	5767.200	6001.200
5	0.48	3	6235.200	6235.200	6001.200
6	0.92	3	3310.400	3310.400	3310.400
7	1.97	3	342.800	342.800	342.800

biomass
File: 6106b 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	6898.800				
0.053	6898.800	0.002		1.74	k= 1, v=17
0.111	6788.400	0.244		1.82	k= 2, v=17
0.24	6001.200	1.996	*	1.85	k= 3, v=17
0.48	6001.200	1.996	*	1.87	k= 4, v=17
0.92	3310.400	7.988	*	1.87	k= 5, v=17
1.97	342.800	14.595	*	1.88	k= 6, v=17

s = 635.145
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	0.43	0.34	0.53	0.047	0.80
EC10	0.51	0.42	0.61	0.040	0.82
EC25	0.67	0.58	0.78	0.030	0.86
EC50	0.92	0.83	1.0	0.021	0.90

Slope = 4.94 Std.Err. = 0.459

Goodness of fit: p = 0.18 based on DF= 4.0 17.

6106B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	6.90e+03	6.67e+03	227.	100.	0.00
0.0530	3.00	6.90e+03	6.67e+03	230.	100.	4.64e-08
0.111	3.00	6.79e+03	6.67e+03	117.	100.	0.000287
0.240	3.00	5.77e+03	6.66e+03	-891.	99.8	0.198
0.480	3.00	6.24e+03	6.13e+03	109.	91.8	8.16
0.920	3.00	3.31e+03	3.33e+03	-21.3	49.9	50.1
1.97	3.00	343.	340.	2.31	5.10	94.9

growth rate
File: 6106g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	224862.106	37477.018	173.019
Within (Error)	17	3682.308	216.606	
Total	23	228544.415		

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

growth rate
File: 6106g 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	575.782	575.782		
2	0.053	578.993	578.993	-0.309	
3	0.111	578.807	578.807	-0.291	
4	0.24	556.803	556.803	1.824	
5	0.48	574.837	574.837	0.091	
6	0.92	528.293	528.293	4.563	*
7	1.97	278.493	278.493	28.567	*

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

growth rate
File: 6106g 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	0.053	3	27.630	4.8	-3.212
3	0.111	3	27.630	4.8	-3.025
4	0.24	3	27.630	4.8	18.978
5	0.48	3	27.630	4.8	0.945
6	0.92	3	27.630	4.8	47.488
7	1.97	3	27.630	4.8	297.288

growth rate
File: 6106g 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	575.782	575.782	577.341
2	0.053	3	578.993	578.993	577.341
3	0.111	3	578.807	578.807	577.341
4	0.24	3	556.803	556.803	565.820
5	0.48	3	574.837	574.837	565.820
6	0.92	3	528.293	528.293	528.293
7	1.97	3	278.493	278.493	278.493

growth rate
File: 6106g 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	577.341				
0.053	577.341	0.150		1.74	k= 1, v=17
0.111	577.341	0.150		1.82	k= 2, v=17
0.24	565.820	0.957		1.85	k= 3, v=17
0.48	565.820	0.957		1.87	k= 4, v=17
0.92	528.293	4.563	*	1.87	k= 5, v=17
1.97	278.493	28.567	*	1.88	k= 6, v=17

s = 14.718
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	0.82	0.71	0.94	0.029	0.87
EC10	0.99	0.88	1.1	0.023	0.89
EC25	1.4	1.3	1.4	0.013	0.94
EC50	1.9	1.9	2.0	0.0065	0.97

Slope = 4.39 Std.Err. = 0.340

Goodness of fit: p = 0.38 based on DF= 4.0 17.

6106G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	576.	574.	1.96	100.	0.00
0.0530	3.00	579.	574.	5.17	100.	3.58e-10
0.111	3.00	579.	574.	4.99	100.	2.59e-06
0.240	3.00	557.	574.	-17.0	100.	0.00351
0.480	3.00	575.	572.	3.29	99.6	0.397
0.920	3.00	528.	529.	-0.409	92.1	7.86
1.97	3.00	278.	278.	0.0349	48.5	51.5

EAD Assessment of USEPA DER

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

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity of the Formulation to Freshwater Algae (green algae, *Pseudokircheneriella subcapitata* (a.k.a. *Selenastrum capricornutum*))

Kern, M.E. and Lam, C.V. 2004. Toxicity of JAU 6476 480 SC to the Green Alga *Pseudokirchneriella subcapitata* (a.k.a. *Selenastrum capricornutum*). Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX075 (J6883501), and sponsored by Bayer CropScience, RTP, NC. Experimental start date September 9, 2002 and experimental termination date September 13, 2002. The final report issued January 23, 2004.

PMRA DATA CODE: 9.8.6
EPA DP Barcode: D303488
OECD Data Point: IIA 8.4.1
EPA MRID: 46246106
EPA Guideline: 123-2 (OPPTS 850.5400)

Reviewing Agency: US EPA

EAD Executive Summary

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to Prothioconazole formulation (JAU 6476 480 SC; purity 43%) under static conditions at nominal concentrations of 0 (negative and formulation blank controls), 0.063, 0.125, 0.25, 0.5, and 1.0 and 2.0 mg a.i./L. The 0-hour measured concentrations were <0.021 (< LOQ, controls), 0.053, 0.111, 0.24, 0.48, 0.92, and 1.97 ppm a.i. (84-98% of nominal), while the day 4 measured concentrations were <0.021, 0.020, 0.066, 0.20, 0.42, 0.83, and 1.78 mg a.i./L (32-89% of nominal). According to U.S. EPA policy, the U.S. EPA reviewer used the 0-hour measured concentrations for toxicity estimates because the test substance declined to less than 70% of nominal for the three lowest test concentrations over the study period. The EAD reviewer finds the values used acceptable, as the differences in results expressed in terms of measured day 0, mean measured or measured day 4 concentrations are small and are unlikely to affect the outcome of risk assessments. The study was conducted following U.S. EPA Guideline 123-3 and in compliance with U.S. EPA (40 CFR, Part 160). The 96-hour cell density percent inhibitions were -3, -3, 17, 1, 37, and 94% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 mg a.i./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 0, 2, 16, 10, 52, and 95% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 mg a.i./L treatment groups,

respectively. The growth rate (0 to 96 hours) percent inhibitions were -1, -1, 3, 0, 8, and 52% in the 0.053, 0.11, 0.24, 0.48, 0.92, and 1.97 mg a.i./L treatment groups, respectively. The NOEC for all endpoints was 0.48 mg a.i./L.The 96-hour EC_{50}/IC_{50} for cell density was 1.1 mg a.i./L (95% C.I.: 0.94-1.2 mg a.i./L), while it was 0.92 mg a.i./L (95% C.I.: 0.83-1.0 mg a.i./L) and 1.9 mg a.i./L (95% C.I.: 1.9-2.0 mg a.i./L) for area under the growth curve and for growth rate, respectively.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type: Static

Cell density:
NOEC: 0.48 mg a.i./L
EC_{50}/IC_{50}: 1.1 mg a.i./L 95% C.I.: 0.94-1.2 mg a.i./L
Slope: 5.88±0.718

Growth rates:
NOEC: 0.48 mg a.i./L
EC_{50}/IC_{50}: 1.9 mg a.i./L 95% C.I.: 1.9-2.0 mg a.i./L
Slope: 4.39±0.340

Plant biomass (area under the growth curve):
NOEC: 0.48 mg a.i./L
EC_{50}/IC_{50}: 0.92 mg a.i./L 95% C.I.: 0.83-1.0 mg a.i./L
Slope: 4.94±0.459

Endpoint(s) Affected: Cell density, growth rates, 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 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.

3. 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 used both the day 0 and the mean measured concentrations to estimate the EC_{50}/IC_{50}. Irrespective of which concentrations were used, results for were within the confidence intervals or were very close to the results reported by the EPA reviewer and the study authors. The slight difference in values is unlikely to result in significant effects on risk assessments. The values reported by the EPA reviewer are therefore acceptable to EAD.

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

Statistical Output of the EAD reviewer

Cell density

```
Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------
Conc. Tested    00.0360.088 0.22 0.450.8751.875
-----------------------------------------------
Response  1   303221.4283.4189.7215.2170.9 12.7
Response  2 271.7263.8280.6219.6248.7  147 16.3
Response  3 215.7298.8218.3221.1289.3161.3 14.7
Response  4 259.7
Response  5 283.7
Response  6 193.5
-----------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole formulation Density Mean
Test Start Date:    Test Ending Date:
Test Species: Pseudokirchneriella subcapitata (S. capricornutum)
Test Duration:        96 hours
DATA FILE: psedenfM.icp
OUTPUT FILE: psedenfM.i50
```

Conc. ID	Number Replicates	Concentration mg ai/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	254.550	41.843	257.800
2	3	0.036	261.333	38.759	257.800
3	3	0.088	260.767	36.804	257.800
4	3	0.220	210.133	17.712	230.600
5	3	0.450	251.067	37.107	230.600
6	3	0.875	159.733	12.027	159.733
7	3	1.875	14.567	1.804	14.567

```
The Linear Interpolation Estimate:     1.0874   Entered P Value: 50
-----------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   1.0776 Standard Deviation:     0.0481
Original Confidence Limits:   Lower:     0.9745 Upper:     1.1564
Expanded Confidence Limits:   Lower:     0.9406 Upper:     1.1771
Resampling time in Seconds:     0.05  Random_Seed: 898427886
```

Biomass (area under the growth curve)

```
Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------
Conc. Tested    00.0360.088 0.22 0.450.8751.875
-----------------------------------------------
Response  1 7958.4 60367084.85278.85599.23337.2301.2
Response  2  71227132.87195.26079.26198.03225.6380.4
Response  3 6078.07533.66085.25943.66908.43368.4346.8
Response  4 6932.4
Response  5 7568.4
Response  6 5727.6
-----------------------------------------------
```

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole formulation biomass mean
Test Start Date:    Test Ending Date:
Test Species: Pseudokirchneriella subcapitata (S. capricornutum)
Test Duration:        96 hours
DATA FILE: psebiofM.icp
OUTPUT FILE: psebiofM.i50
```

Conc. ID	Number Replicates	Concentration mg ai/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	6897.800	856.444	6898.800
2	3	0.036	6900.800	775.287	6898.800
3	3	0.088	6788.400	611.486	6788.400
4	3	0.220	5767.200	428.366	6001.200
5	3	0.450	6235.200	655.392	6001.200
6	3	0.875	3310.400	75.078	3310.400
7	3	1.875	342.800	39.751	342.800

```
The Linear Interpolation Estimate:     0.8530   Entered P Value: 50
-------------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   0.8473 Standard Deviation:     0.0246
Original Confidence Limits:   Lower:     0.8094 Upper:     0.8942
Expanded Confidence Limits:   Lower:     0.7963 Upper:     0.9066
Resampling time in Seconds:     0.00  Random_Seed: 1566257262
```

Growth rate

```
Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------
Conc. Tested    00.0360.088 0.22 0.450.8751.875
-----------------------------------------------
Response  1 0.0595180.0562500.0588210.0546400.0559540.0535530.026475
Response  2 0.0583820.0580750.0587180.0561650.0574610.0519840.029075
Response  3 0.0559780.0593730.0561030.0562360.0590360.0529510.027998
Response  4 0.057912
Response  5 0.058832
Response  6 0.054847
-----------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole formulation growth mean
Test Start Date:    Test Ending Date:
Test Species: Pseudokirchneriella subcapitata (S. capricornutum)
Test Duration:        96 hours
DATA FILE: psegrofM.icp
OUTPUT FILE: psegrofM.i50
```

Conc. ID	Number Replicates	Concentration mg ai/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.058	0.002	0.058
2	3	0.036	0.058	0.002	0.058
3	3	0.088	0.058	0.002	0.058
4	3	0.220	0.056	0.001	0.057

5	3	0.450	0.057	0.002	0.057
6	3	0.875	0.053	0.001	0.053
7	3	1.875	0.028	0.001	0.028

The Linear Interpolation Estimate: 1.8343 Entered P Value: 50

Number of Resamplings: 80Those resamples not used had estimates above the highest concentration/ %Effluent.

The Bootstrap Estimates Mean: 1.8304 Standard Deviation: 0.0211

No Confidence Limits can be produced since the number of resamples generated is not a multiple of 40.
Resampling time in Seconds: 0.00 Random_Seed: -2133066258