Data Evaluation Report on the acute toxicity of Prothioconazole on the Algae, *Selenastrum capricornutum*
PMRA Submission #:2004-0843 EPA MRID #: 46246105

Data Requirement:

PMRA DATA CODE	9.8.2
EPA DP Barcode	D303488
OECD Data Point	IIA 8.4.1
EPA MRID	46246105
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: JAU 6476 **Purity:** 98.3%
Common name: Prothioconazole
Chemical name: IUPAC:3H-1,2,4,-Triazole-3-thione, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro
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: JAU 6476

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 8/18/04

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

Signature:
Date: 8/26/04

Primary Reviewer: Kevin Costello
EPA/OPP/EFED/ERB-III

Date: {.....}

Secondary Reviewer(s): Christopher J. Salice
EPA/OPP/EFED/ERB-IV

Date: 9/15/2005
9-15-05

Secondary Reviewer: Émilie Larivière
HC, PMRA, EAD

Date: 11/2/2005
11/2/05

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

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Dorgerloh, M. 2000. JAU6476 Influence on the Growth of the Green Alga, *Selenastrum capricornutum*. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany, Laboratory Study No. E 3231610-6, and sponsored by Bayer CropScience, RTP, NC. Experimental start date October 15, 1999 and experimental termination date January 21, 2000. The final report issued October 25, 2000.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0.098, 0.197, 0.393, 0.786, 1.57, and 3.15 ppm a.i.. The 0-hour measured concentrations were 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 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 three lowest test concentrations over the study period. The 96-hour cell density percent inhibitions were -24.80, -18.09, -3.78, 35.03, 81.21, and 97.02% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were -22.1, -15.9, -1.3, 33.4, 77.1, and 95.2% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -3.9, -2.9, -0.6, 7.9, 30.6, and 64.1% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i. treatment groups, respectively. The NOAEC was 0.371 ppm a.i. for all endpoints. Cell density was the most sensitive endpoint tested; the **96-hour** EC_{50} was 0.88 ppm a.i..

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

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell density; reviewer-reported (96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{05}: 0.32 ppm a.i. 95% C.I.: 0.25-0.42 ppm a.i.
EC_{50}/IC_{50}: 0.88 ppm a.i. 95% C.I.: 0.78-1.0 ppm a.i.
Slope: 3.76±0.322

Growth rates; study author-reported (96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{05}: not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 2.34 ppm a.i. 95% C.I.: 2.28-2.40 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve; study author-reported; 96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{05}: not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 1.05 ppm a.i. 95% C.I.: 0.99-1.13 ppm a.i.
Slope: N/A

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218, and U.S. EPA-FIFRA Guideline 123-2,Tier 2 (and OPPTS Guideline Number 850.5400). The following deviations from U.S. EPA Guideline, §123-2 were noted:

1. The dilution water characteristics were not reported.
2. The agitation rate of 3 rpm was less than recommended (100 rpm).
3. The light intensity of 8 klux was greater than the recommended 4-5 klux.

These deviations did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice (Chemicals Law (Chem G), dated July 25, 1994, Annex 1, and the OECD Principles of Good Laboratory Practice (1997), (pp. 1c and 2).

A. MATERIALS:

1. Test Material Prothioconazole (JAU 6476 Technical)

Description: White crystals

Lot No./Batch No. : Fl. 6233/0031

Purity: 98.3%

Stability of Compound Under Test Conditions: The 0 hour measured test concentrations were 87-97% of the nominal concentrations and the 96 hour measured test concentrations were 36-94% of the nominal concentrations. In the three 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: 89 mg/L (23°C, pH 7)

Hydrolytic Stability: t½: >500h (50°C, pH 4-9)

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Name: *Selenastrum capricornutum*

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: SAG 61.81
Source: Collection of Algal Cultures, Institute for Plant Physiology, University of Gottingen, Gottingen, Germany.
Age of inoculum: 3 days old
Method of cultivation: Nutrient Solution 2 (p. 7)

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range finding study was reported.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous	
culturing media and conditions: (same as test or not)	Nutrient Solution 1 (p. 7); this culture nutrient solution was modified for testing (Nutrient solution 2).	*EPA recommends two week acclimation period.*
health: (any toxicity observed)	Not reported.	*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	Incubator	

Parameter	Details	Remarks *Criteria*
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	 Erlenmeyer flasks 300 mL 150 mL	The test vessels were sealed with cotton wool plugs. *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):**	 Nutrient Solution 2 7.98-8.20 8.46-9.66 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)	Yes (p. 7)	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water Sterile Not reported N/A None Not reported Not reported Not reported Not reported Not reported	The dilution water characteristics were not reported. *EPA pH: <u>Skeletonema costatum</u>= ~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	

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Agitation, 3 rpm	The agitation rate of 3 rpm was less than recommended (100 rpm). *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*
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:	 6 6 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.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative and solvent controls), 0.098, 0.197, 0.393, 0.786, 1.57, and 3.15 ppm a.i. (corresponding to 0.10, 0.20, 0.40, 0.80, 1.60, and 3.20 mg/L) Day 0: 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i. Day 4: 0.035, 0.089, 0.235, 0.628, 1.36, and 2.97 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)	Dimethylformamide, concentration not reported	
Method and interval of analytical verification	HPLC; 0 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	23±2°C Continuous 8000 lux (±20%), fluorescent lights.	The light intensity of 8 klux was greater than the recommended 4-5 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:	Potassium dichromate ($K_2Cr_2O_7$) 0.10, 0.18, 0.32, 0.56, 1.00, and 1.80 mg/L	This test was conducted on May 21, 1999. The 0-72 hour biomass EC_{50} was 0.71 mg/L. The 0-72 hour growth rate EC_{50} was 1.39 mg/L.
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, growth rate, and area under the growth curve (biomass).	*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.*
Measurement technique for cell density and other end points	Counting chamber and microscope.	Photometer used for estimations. *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 222-224X 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?	Cell density replicate data were provided; biomass and growth rate replicate data were not.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 96-hour cell density percent inhibitions were -24.80, -18.09, -3.78, 35.03, 81.21, and 97.02% in the 0.086, 0.182,

0.371, 0.747, 1.52, and 3.03 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were -22.1, -15.9, -1.3, 33.4, 77.1, and 95.2% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -3.9, -2.9, -0.6, 7.9, 30.6, and 64.1% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i. treatment groups, respectively.

Table 3: Effect of Prothioconazole on Algae (*Selenastrum capricornutum*)

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	60,600	2,237,000	--
Solvent control	10,000	61,600	2,248,500	--
0.086 (0.098)	10,000	66,600	2,791,700	-24.80
0.182 (0.197)	10,000	66,600	2,641,700	-18.09
0.371 (0.393)	10,000	64,700	2,321,700	-3.78
0.747 (0.786)	10,000	54,100	1,453,300	35.03*
1.52 (1.57)	10,000	43,400	420,300	81.21*
3.03 (3.15)	10,000	29,100	66,700	97.02*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The 96 hour cell densities and percent inhibitions were obtained from Table 6c, p. 18.
* Significantly different from the control (Dunnett's test).

Table 4: Effect of Prothioconazole on Algae (*Selenastrum capricornutum*)

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	1.37	0.0	5451	0.0
0.086 (0.098)	10,000	1.43	-3.9	6657	-22.1
0.182 (0.197)	10,000	1.41	-2.9	6317	-15.9
0.371 (0.393)	10,000	1.38	-0.6	5523	-1.3

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]
0.747 (0.786)	10,000	1.26	7.9*	3630	33.4*
1.52 (1.57)	10,000	0.95	30.6*	1250	77.1*
3.03 (3.15)	10,000	0.49	64.1*	262	95.2*
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The values were based on 0-96 hour data. The statistical analyses used 0-72 hour data.
* Significantly different from the control (Tables 6a-6b, pp. 16-17).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density
NOAEC or EC_{05} (ppm a.i.)	0.371	0.371	0.371
EC_{50} (ppm a.i.)	1.10	2.18	1.05
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	0.84-1.43	2.08-2.29	0.747-1.52
Reference chemical, if used NOAEC EC_{50}	 Not reported 0.71 mg/L	 Not reported 1.39 mg/L	 Not reported

[a] The biomass and growth rate statistics based on 0-72 hour data.

B. REPORTED STATISTICS:

Statistical Method: The NOAEC and LOAEC values were determined using analysis of variance (Dunnett's-Test). The EC_{50} values for biomass and growth rate (0-72 hours) were calculated using probit analysis. The EC_{50} for cell density was based on 96 hour cell densities. The statistical analyses were conducted using the commercial software (Ratte, 1993-1998). All statistical calculations were performed using the initial (Day 0) measured concentrations.

Cell density (96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{50}/IC_{50}: 1.05 ppm a.i. 95% C.I.: 0.747-1.52 ppm a.i.
Slope: N/A

Growth rates (72-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{50}/IC_{50}: 2.18 ppm a.i. 95% C.I.: 2.08-2.29 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve; 72-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{50}/IC_{50}: 1.10 ppm a.i. 95% C.I.: 0.84-1.43 ppm a.i.
Slope: N/A

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density data were square-root transformed to satisfy the assumptions of ANOVA (i.e., normality and homogeneity of variances). The NOAEC and LOAEC were determined using ANOVA, followed by William's multiple comparison test. 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. Replicate data for growth rate and biomass were not provided, so results for these endpoints could not be statistically verified.

Cell density:
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{05}: 0.32 ppm a.i. 95% C.I.: 0.25-0.42 ppm a.i.
EC_{50}/IC_{50}: 0.88 ppm a.i. 95% C.I.: 0.78-1.0 ppm a.i.
Slope: 3.76±0.322

Endpoint(s) Affected: Cell density
Most sensitive endpoint: Cell density

D. STUDY DEFICIENCIES:

There were no significant study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study author's; the reviewer's EC_{50} estimate for cell density was slightly lower than the study author's, but it was the most sensitive endpoint (based on the study author-reported EC_{50} estimates for the other endpoints). The reviewer calculated an EC_{05} value for cell density for the purpose of endangered species risk assessment. The reviewer's estimates for cell density are associated with a slope value so they were 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 was the most sensitive endpoint, with an EC_{50} of 0.88 ppm a.i..

Cell density; reviewer-reported (96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.

EC_{05}: 0.32 ppm a.i. 95% C.I.: 0.25-0.42 ppm a.i.
EC_{50}/IC_{50}: 0.88 ppm a.i. 95% C.I.: 0.78-1.0 ppm a.i.
Slope: 3.76±0.322

Growth rates; study author-reported (96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{05}: not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 2.34 ppm a.i. 95% C.I.: 2.28-2.40 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve; study author-reported; 96-hour):
NOAEC: 0.371 ppm a.i.
LOAEC: 0.747 ppm a.i.
EC_{05}: not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 1.05 ppm a.i. 95% C.I.: 0.99-1.13 ppm a.i.
Slope: N/A

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

III. REFERENCES:

ASTM-Guideline No. E 1218-90 (1990), "Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae" (August 1990)

Bringmann, G. and Kuhn, R. (1980). Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibition test. Water Research 14: 231-241

Dorgerloh, M. (1999). Influence of Potassium Dichromate (Reference Chemical) on the Growth of the Green Alga, *Selenastrum capricornutum*. Unpublished Research Report of the Bayer AG, No. DOM 99004, May 28, 1999.

EEC Directive 79/831/E, Annex V, C.3, Algal Inhibition Test, Revised Version NO. L 383 A/179 (29 Dec. 1992)

Finney, D.J. (1952). "Statistical Methods in Biological Assay", London

GLP Standards (Chemicals Law (ChemG) of July 25, 1994, Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]).

ISO-Guideline NO. 8692: 1989 (E) "Water Quality-Fresh Water Algal Growth Inhibition Test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*" (15 Nov. 1989)

Litchfield, J.F. and Wilcoxon, F.A. (1949). A simplified method of evaluating dose-effect experiments. J. Pharmacol. 31: 99-113

Nygaard, G.; J. Komarek, J. Kristiansen & O.M. Skulberg (1986): taxonomic designation of the bioassay alga NIVA-CHL 1 ("Selenastrum capricornutum") and some related strains. Opera Botanica 90: 5-46 (1986).

OECD-Guideline No. 201 (1984), "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (7 June 1984).

Ratte (1998), "Easy Assay, Algae Growth Inhibition", Version 4.01, 1993-1998, SpiRiT Aachen, 52066 Aachen,

Germany.

U.S. EPA-FIFRA,.Subdivision N, § 123-2 "Growth and Reproduction of Aquatic Plants, Tier 2"

U.S. EPA-Guideline 540/9-86-134 (Environmental Protection Agency, Hazard Evaluation Division, Standard Evaluation Procedure-Non-Target Plants Growth and Reproduction of Aquatic Plants, Tiers 1 and 2, June 1986).

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6105cd Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	589.659	98.277	283.219
Within (Error)	23	7.984	0.347	
Total	29	597.643		

Critical F value = 2.53 (0.05,6,23)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 6105cd 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	14.960	224.275		
2	0.086	16.699	279.167	-4.574	
3	0.182	16.245	264.167	-3.382	
4	0.371	15.236	232.167	-0.728	
5	0.747	12.055	145.333	7.639	*
6	1.52	6.475	42.033	22.314	*
7	3.03	2.580	6.667	32.559	*

Bonferroni T table value = 2.58 (1 Tailed Value, P=0.05, df=23,6)

cell density
File: 6105cd 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	12			
2	0.086	3	28.421	12.7	-54.892
3	0.182	3	28.421	12.7	-39.892
4	0.371	3	28.421	12.7	-7.892
5	0.747	3	28.421	12.7	78.942
6	1.52	3	28.421	12.7	182.242
7	3.03	3	28.421	12.7	217.608

cell density
File: 6105cd 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	12	224.275	14.960	15.464
2	0.086	3	279.167	16.699	15.464
3	0.182	3	264.167	16.245	15.464
4	0.371	3	232.167	15.236	15.236
5	0.747	3	145.333	12.055	12.055
6	1.52	3	42.033	6.475	6.475
7	3.03	3	6.667	2.580	2.580

cell density
File: 6105cd 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	15.464				
0.086	15.464	1.326		1.72	k= 1, v=23
0.182	15.464	1.326		1.80	k= 2, v=23
0.371	15.236	0.727		1.83	k= 3, v=23
0.747	12.055	7.638	*	1.84	k= 4, v=23
1.52	6.475	22.309	*	1.85	k= 5, v=23
3.03	2.580	32.552	*	1.85	k= 6, v=23

s = 0.589
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	0.32	0.25	0.42	0.058	0.76
EC10	0.40	0.32	0.51	0.050	0.79
EC25	0.58	0.49	0.70	0.038	0.84
EC50	0.88	0.78	1.0	0.026	0.89

Slope = 3.76 Std.Err. = 0.322

!!!Poor fit: p < 0.001 based on DF= 4.00 23.0

6105CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	12.0	224.	241.	-17.1	100.	0.00
0.0860	3.00	279.	241.	37.8	100.	0.00711
0.182	3.00	264.	240.	24.0	99.5	0.495
0.371	3.00	232.	222.	9.71	92.2	7.85
0.747	3.00	145.	147.	-1.21	60.7	39.3
1.52	3.00	42.0	45.1	-3.09	18.7	81.3
3.03	3.00	6.67	5.28	1.39	2.19	97.8

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: November 2, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Freshwater Algae (green algae, *Selenastrum capricornutum*)

Dorgerloh, M. 2000. JAU6476 Influence on the Growth of the Green Alga, *Selenastrum capricornutum*. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany, Laboratory Study No. E 3231610-6, and sponsored by Bayer CropScience, RTP, NC. Experimental start date October 15, 1999 and experimental termination date January 21, 2000. The final report issued October 25, 2000.

PMRA DATA CODE: 9.8.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.4.1
EPA MRID: 46246105
EPA Guideline: 123-2 (OPPTS 850.5400)

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Prothioconazole (JAU 6476 Technical, purity 98.3%) under static conditions at nominal concentrations of 0.098, 0.197, 0.393, 0.786, 1.57, and 3.15 mg a.i./L. The study was conducted in accordance with EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218, and U.S. EPA-FIFRA Guideline 123-2,Tier 2 (and OPPTS Guideline Number 850.5400), and was in compliance with German and OECD Principles of GLP. The 0-hour measured concentrations were 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 mg a.i./L, while the day-4 measured concentrations were 0.035, 0.089, 0.235, 0.628, 1.36, and 2.97 mg a.i./L. 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 96-hour cell density percent inhibitions were -24.80, -18.09, -3.78, 35.03, 81.21, and 97.02% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03mg a.i./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were -22.1, -15.9, -1.3, 33.4, 77.1, and 95.2% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 mg a.i./L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -3.9, -2.9, -0.6, 7.9, 30.6, and 64.1% in the 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 mg a.i./L treatment

groups, respectively. The NOEC was 0.371 mg a.i./L for all endpoints. The 96-hour EC_{50}/IC_{50} for cell density was 0.88 mg a.i./L (95% C.I.: 0.78-1.0 mg a.i./L), while it was 1.05 mg a.i./L (95% C.I.: 0.99-1.13 mg a.i./L) and 2.34 mg a.i./L (95% C.I.: 2.28-2.40 mg a.i./L) for area under the growth curve and for growth rate, respectively.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell density; EPA reviewer-reported (96-hour):
NOEC: 0.371 mg a.i./L
LOEC: 0.747 mg a.i./L
EC_{50}/IC_{50}: 0.88 mg a.i./L 95% C.I.: 0.78-1.0 mg a.i./L
Slope: 3.76±0.322

Growth rates; EAD reviewer-reported (96-hour):
NOEC: 0.371 mg a.i./L
LOEC: 0.747 mg a.i./L
EC_{50}/IC_{50}: 2.34 mg a.i./L 95% C.I.: 2.28-2.40 mg a.i./L
Slope: N/A

Plant biomass (area under the growth curve; EAD reviewer-reported, 96-hour):
NOEC: 0.371 mg a.i./L
LOEC: 0.747 mg a.i./L
EC_{50}/IC_{50}: 1.05 mg a.i./L 95% C.I.: 0.99-1.13 mg a.i./L
Slope: N/A

Endpoint(s) Affected: Cell density, growth rates, 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 results very close to those reported by the study author. 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 more conservative day-4 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. 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. The EAD reviewer also used the replicate data for biomass and growth rate calculated from cell density to estimate the 96-hour EC_{50}/IC_{50}s for those two endpoints. The percent inhibition observed at 72 and at 96 hours are very similar and the difference in the 72 versus 96-hour EC_{50}/IC_{50}s is minor. Results for the 0-96 hour period are reported for the sake of consistency, as percent inhibition at 96 hour is reported in the Executive Summary.

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 concurs with the NOECs determined by the study author and of the EPA reviewer. The small difference in concentrations between using the day-0 measured values, the day-4 measured values or the mean measured values to determine the NOEC is unlikely to affect the outcome of a risk assessment. The values reported by the EPA reviewer (day 0) are therefore acceptable.

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

Statistical verification of the EAD reviewer

Cell density

```
Conc. ID         1     2     3     4     5     6     7
-------------------------------------------------------
Conc. Tested     00.0860.1820.3710.747 1.52 3.03
-------------------------------------------------------
Response  1    272305.8253.2232.9147.6 48.1  7.5
Response  2    208269.4251.8237.2  147 38.8  6.2
Response  3 228.8262.3287.5226.4141.4 39.2  6.3
Response  4 209.8
Response  5 191.1
Response  6 232.5
Response  7 196.6
Response  8 241.9
Response  9 237.2
Response 10 232.5
Response 11 218.5
Response 12 222.4
-----------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole (JAU6476) Cell density
Test Start Date:     Test Ending Date:
Test Species: S. capricornutum  Pooled controls, day 0 measured
Test Duration:         96 hours
DATA FILE: scdenp0.icp
OUTPUT FILE: scdenp0.i50
```

Conc. ID	Number Replicates	Concentration mg ai/L	Response Means	Std. Dev.	Pooled Response Means
1	12	0.000	224.275	21.927	240.072
2	3	0.086	279.167	23.337	240.072
3	3	0.182	264.167	20.219	240.072
4	3	0.371	232.167	5.437	232.167
5	3	0.747	145.333	3.420	145.333
6	3	1.520	42.033	5.258	42.033
7	3	3.030	6.667	0.723	6.667

```
The Linear Interpolation Estimate:     0.9363   Entered P Value: 50
-------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   0.9387 Standard Deviation:     0.0215
Original Confidence Limits:   Lower:     0.8995 Upper:     0.9817
Expanded Confidence Limits:   Lower:     0.8921 Upper:     0.9908
Resampling time in Seconds:     0.00  Random_Seed: 253471270
```

Growth rate

```
Conc. ID         1     2     3     4     5     6     7
-------------------------------------------------------
Conc. Tested     00.0860.1820.3710.747 1.52 3.03
-------------------------------------------------------
Response  1 0.0583940.0596140.0576480.0567770.0520260.0403470.020989
Response  2 0.0555990.0582940.057590.0579680.0519840.0381090.019006
```

```
Response  3 0.0565920.0580160.0589710.0564820.0515790.0382150.019172
Response  4 0.055689
Response  5 0.054717
Response  6 0.056759
Response  7 0.055012
Response  8 0.057172
Response  9 0.056968
Response 10 0.056759
Response 11 0.056112
Response 12 0.056297
-----------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole (JAU6476) Growth rate
Test Start Date:    Test Ending Date:
Test Species: S. capricornutum  Pooled controls, day 0 measured
Test Duration:        96 hours
DATA FILE: scgrop0.icp
OUTPUT FILE: scgrop0.i50
-------------------------------------------------------------------------
Conc.      Number      Concentration      Response      Std.        Pooled
 ID      Replicates          mg ai/L        Means        Dev.    Response Means
-------------------------------------------------------------------------
  1         12              0.000          0.056       0.001       0.057
  2          3              0.086          0.059       0.001       0.057
  3          3              0.182          0.058       0.001       0.057
  4          3              0.371          0.057       0.001       0.057
  5          3              0.747          0.052       0.000       0.052
  6          3              1.520          0.039       0.001       0.039
  7          3              3.030          0.020       0.001       0.020
-------------------------------------------------------------------------
The Linear Interpolation Estimate:     2.3377   Entered P Value: 50
-------------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   2.3389 Standard Deviation:     0.0303
Original Confidence Limits:   Lower:     2.2822 Upper:     2.4007
Expanded Confidence Limits:   Lower:     2.2711 Upper:     2.4133
Resampling time in Seconds:     0.05  Random_Seed: 2053748518

Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------
Conc. Tested    00.0860.1820.3710.747 1.52 3.03
-----------------------------------------------
Response  1 0.0583940.0596140.0576480.0567770.0520260.0403470.020989
Response  2 0.0555990.0582940.057590.0579680.0519840.0381090.019006
Response  3 0.0565920.0580160.0589710.0564820.0515790.0382150.019172
Response  4 0.055689
Response  5 0.054717
Response  6 0.056759
Response  7 0.055012
Response  8 0.057172
Response  9 0.056968
Response 10 0.056759
Response 11 0.056112
Response 12 0.056297
-----------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole (JAU6476) Growth rate
Test Start Date:    Test Ending Date:
```

```
Test Species: S. capricornutum  Pooled controls, day 0 measured
Test Duration:        96 hours
DATA FILE: scgrop0.icp
OUTPUT FILE: scgrop0.i05
```

Conc. ID	Number Replicates	Concentration mg ai/L	Response Means	Std. Dev.	Pooled Response Means
1	12	0.000	0.056	0.001	0.057
2	3	0.086	0.059	0.001	0.057
3	3	0.182	0.058	0.001	0.057
4	3	0.371	0.057	0.001	0.057
5	3	0.747	0.052	0.000	0.052
6	3	1.520	0.039	0.001	0.039
7	3	3.030	0.020	0.001	0.020

```
The Linear Interpolation Estimate:     0.5789   Entered P Value:  5
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   0.5748 Standard Deviation:     0.0128
Original Confidence Limits:   Lower:     0.5511 Upper:     0.5953
Expanded Confidence Limits:   Lower:     0.5455 Upper:     0.5986
Resampling time in Seconds:     0.06  Random_Seed: 2110800070
```

Biomass (area under the growth curve)

```
Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------
Conc. Tested    00.0860.1820.3710.747 1.52 3.03
-----------------------------------------------
Response  1 6580.87341.66007.25535.6 36241443.6274.8
Response  2 5131.26403.26129.65491.2 36361137.6230.4
Response  3 5740.8 62106795.6 55203609.6 1152262.8
Response  4 4804.8
Response  5 4827.6
Response  6  5538
Response  7 4675.2
Response  8  5682
Response  9 6055.2
Response 10  2778
Response 11  2610
Response 12 2656.8
-----------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole (JAU6476) Biomass
Test Start Date:    Test Ending Date:
Test Species: S. capricornutum  Pooled controls, day 0 measured
Test Duration:        96 hours
DATA FILE: scbiop0.icp
OUTPUT FILE: scbiop0.i50
```

Conc.	Number	Concentration	Response	Std.	Pooled

ID	Replicates	mg ai/L	Means	Dev.	Response Means
1	12	0.000	4756.700	1366.345	5357.829
2	3	0.086	6651.600	605.315	5357.829
3	3	0.182	6310.800	424.286	5357.829
4	3	0.371	5515.600	22.525	5357.829
5	3	0.747	3623.200	13.218	3623.200
6	3	1.520	1244.400	172.662	1244.400
7	3	3.030	256.000	22.968	256.000

The Linear Interpolation Estimate: 1.0538 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 1.0589 Standard Deviation: 0.0396
Original Confidence Limits: Lower: 0.9868 Upper: 1.1323
Expanded Confidence Limits: Lower: 0.9734 Upper: 1.1480
Resampling time in Seconds: 0.00 Random_Seed: 253471270

Conc. ID	1	2	3	4	5	6	7
Conc. Tested	0	0.086	0.182	0.371	0.747	1.52	3.03
Response 1	6580.8	7341.6	6007.2	5535.6	3624	1443.6	274.8
Response 2	5131.2	6403.2	6129.6	5491.2	3636	1137.6	230.4
Response 3	5740.8	6210	6795.6	5520	3609.6	1152	262.8
Response 4	4804.8						
Response 5	4827.6						
Response 6	5538						
Response 7	4675.2						
Response 8	5682						
Response 9	6055.2						
Response 10	2778						
Response 11	2610						
Response 12	2656.8						

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole (JAU6476) Biomass
Test Start Date: Test Ending Date:
Test Species: S. capricornutum Pooled controls, day 0 measured
Test Duration: 96 hours
DATA FILE: scbiop0.icp
OUTPUT FILE: scbiop0.i05

Conc. ID	Number Replicates	Concentration mg ai/L	Response Means	Std. Dev.	Pooled Response Means
1	12	0.000	4756.700	1366.345	5357.829
2	3	0.086	6651.600	605.315	5357.829
3	3	0.182	6310.800	424.286	5357.829
4	3	0.371	5515.600	22.525	5357.829
5	3	0.747	3623.200	13.218	3623.200
6	3	1.520	1244.400	172.662	1244.400
7	3	3.030	256.000	22.968	256.000

The Linear Interpolation Estimate: 0.4291 Entered P Value: 5

Number of Resamplings: 80
The Bootstrap Estimates Mean: 0.4228 Standard Deviation: 0.0249

```
Original Confidence Limits:   Lower:     0.3292 Upper:     0.4422
Expanded Confidence Limits:   Lower:     0.3092 Upper:     0.4449
Resampling time in Seconds:     0.05  Random_Seed: 1595519942
```