Data Evaluation Report on the acute toxicity of Prothioconazole on the Algae, *Navicula pelliculosa*
PMRA Submission #: 2004-0843 EPA MRID #: 46246109

Data Requirement:

PMRA DATA CODE	9.8.2
EPA DP Barcode	D303488
OECD Data Point	IIA 8.4.1
EPA MRID	46246109
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: JAU 6476 Technical **Purity:** 97.5%
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.: 178928-70-6
Synonyms: JAU6476

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11/2/05

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 Technical to the Freshwater Diatom *Navicula pelliculosa*. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX085 (J6883401), and sponsored by Bayer CropScience, RTP, NC. Experimental start date April 22, 2002 and experimental termination date April 26, 2002. The final report issued March 12, 2004.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Navicula pelliculosa* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0 (negative and solvent controls), 26.0, 64.0, 160.0, 400.0, and 1000.0 ppb a.i.. The 0-hour measured concentrations were <2.6 (< LOQ, controls), 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i.; the 0-hour measured concentrations were used for toxicity estimates. The 96-hour cell density percent inhibitions were 18, 11, 31, 74, and 100% in the 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 16, 17, 43, 83, and 101% in the 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 4, 2, 7, 26, and 140% in the 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. treatment groups, respectively. The NOAEC was <23.5 ppb a.i., the lowest test concentration, for cell density and biomass. Biomass (area under the growth curve) was the most sensitive endpoint tested, with an EC_{50} of 180 ppb a.i..

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

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type: Static

Cell density:
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 98 ppb a.i. 95% C.I.: 72-130 ppb a.i.
EC_{50}/IC_{50}: 240 ppb a.i. 95% C.I.: 210-280 ppb a.i.
Slope: 4.16±0.436

Growth rates:
NOAEC: 146.3 ppb a.i.
LOAEC: 356.4 ppb a.i.
EC_{05}: 294.5 ppb a.i. 95% C.I.: 236.4-352.5 ppb a.i. (Study-author reported)
EC_{50}/IC_{50}: 395.3 ppb a.i. 95% C.I.: 317.4-473.2 ppb a.i. (Study-author reported)
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 60 ppb a.i. 95% C.I.: 47-77 ppb a.i.
EC_{50}/IC_{50}: 180 ppb a.i. 95% C.I.: 160-200 ppb a.i.
Slope: 3.45±0.239

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 deviations from U.S. EPA Guideline, §123-2 were noted:

1. The dilution water characteristics were not reported.
2. The test vessel fill volume (50 mL) was less than recommended (100 mL).

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: 97.5%

Stability of Compound Under Test Conditions: The 0 hour measured test concentrations were 89-91% of the nominal concentrations and the 96 hour measured test concentrations were not detected and 13-72% of the nominal concentrations. In all but the highest test level, the 96-hour measured concentration declined <70% of nominal.

(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: *Navicula pelliculosa*

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: NP-78
Source: Originally from University of Texas, Austin, Texas. Current in-house laboratory cultures.
Age of inoculum: 3 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 0.11, 0.33, 1.0, 3.0, and 9.0 µg a.i./L with negative and solvent controls. The percent growth inhibitions were approximately 17, 51, 100, 100, and 100% in the 0.11, 0.33, 1.0, 3.0, and 9.0 µg a.i./L treatment groups, respectively, compared to the pooled controls.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Continuous 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 50 mL	Test vessels covered with inverted glass beakers. The test vessel fill volume was less than recommended. *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 7.2-7.5 6.5-7.4 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 (0.45 µm). 7.5 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:	 4 4 4	*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), 26.0, 64.0, 160.0, 400.0, and 1000.0 ppb a.i. Day 0: <2.6 (< LOQ, controls), 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. Day 4: <2.6 (< LOQ, controls), <2.6, 8.3, 34.8, 206.2, 720.7 ppb a.i.	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Acetone, 0.5 mL/L	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification	HPLC/MS; 0 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	24.4-25.3°C Continuous 4.4 klux	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count (area under the growth curve and growth rates were calculated).	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks/Criteria
Measurement technique for cell density and other end points	Cell counts using a microscope and a hemocytometer.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water control and solvent control cell densities at test termination were 205-208X greater than the dilution water control and solvent control cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 96-hour cell density percent inhibitions were 18, 11, 31, 74, and 100% in the 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 16, 17, 43, 83, and 101% in the 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 4, 2, 7, 26, and 140% in the 23.5, 56.6, 146.3, 356.4, and 889.5 ppb a.i. treatment groups, respectively.

Table 3: Effect of Prothioconazole on Algae (*Navicula pelliculosa*)

Treatment day 0 measured and nominal concentrations [a] (ppb a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	9,000	2,082,000	--
Solvent control	10,000	6,000	2,047,000	--
23.5 (26.0)	10,000	7,000	1,702,000	18*
56.6 (64.0)	10,000	6,000	1,828,000	11*
146.3 (160.0)	10,000	5,000	1,424,000	31*
356.4 (400.0)	10,000	5,000	532,000	74*
889.5 (1000.0)	10,000	6,000	1,300	100*
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.
* Statistically significant from the pooled control (Dunnett's one tailed test).

Table 4: Effect of Prothioconazole on Algae *(Navicula pelliculosa)*

Treatment day 0 measured and Concentrations [a] (ppb a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	--	--	--	--
Solvent control	10,000	--	--	--	--
Pooled control	--	0.05547	--	5341.8	--
23.5 (26.0)	10,000	0.05349	4**	4503.6	16*
56.6 (64.0)	10,000	0.05424	2**	4448.7	17*
146.3 (160.0)	10,000	0.05160	7*	3019.2	43*
356.4 (400.0)	10,000	0.04107	26*	884.1	83*
889.5 (1000.0)	10,000	-0.02218	140*	-42.9	101*
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).
** Statistically significant, but not biologically significant, from the pooled control (Dunnett's one tailed test).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC (ppb a.i.)	<23.5	56.6[1]	<23.5
EC_{05} (ppb a.i.)	42.0	294.5	59.2
EC_{50} (ppb a.i.)	163.8	395.3	215.0
IC_{50} or EC_{50} (ppb a.i.) (95% C.I.)	152.6-175.1	317.4-473.2	193.1-237.0
IC_{25}/EC_{25} (ppb a.i.) (and 95% C.I.)	98.5 (87.4-109.7)	354.2 (284.4-424.0)	132.9 (110.8-155.1)
Reference chemical, if used NOAEC IC_{25}/EC_{25}	N/A	N/A	N/A

N/A = Not applicable.
[1]Based on lack of biologically significant effect at the 23.5 and 56.6 ppb a.i. treatment levels (p. 17).

B. REPORTED STATISTICS:

Statistical Method: The formulas for growth rate and area under the growth curve (biomass) are found on page 50. 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 could not be determined from analyzed data, so an EC_{05} value was estimated. The EC values were determined using the logistic regression model. The parametric analyses were conducted for biomass and nonparametric analyses were conducted for cell density and growth rates. The analyses were conducted using the computer program SAS version 8.2. All statistical calculations were performed using the day 0 measured concentrations.

Cell density:
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 59.2 ppb a.i. 95% C.I.: 40.9-77.5 ppb a.i.
EC_{50}/IC_{50}: 215.0 ppb a.i. 95% C.I.: 193.1-237.0 ppb a.i.
Slope: N/A

Growth rates:

NOAEC:56.6 ppb a.i. (Based on lack of biologically significant effect at the 23.5 and 56.6 ppb a.i. treatment levels)
LOAEC:146.3 ppb a.i.
EC_{05}: 294.5 ppb a.i. 95% C.I.: 236.4-352.5 ppb a.i.
EC_{50}/IC_{50}: 395.3 ppb a.i. 95% C.I.: 317.4-473.2 ppb a.i.
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 42.0 ppb a.i. 95% C.I.: 32.9-51.0 ppb a.i.
EC_{50}/IC_{50}: 163.8 ppb a.i. 95% C.I.: 152.6-175.1 ppb a.i.
Slope: N/A

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density and area under the growth curve (biomass) satisfied the assumptions of ANOVA as determined, however, growth rate data did not satisfy the assumptions of ANOVA (i.e., normality and homogeneity of variances) and transformations were not successful. Non-parametric analyses (Kruskal-Wallis, followed by Dunn's multiple comparison test) were used to determine the NOAEC and LOAEC for growth rate. 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 for cell density and biomass were determined using the Probit method via Nuthatch statistical software; these values could not be determined for growth rate.

Cell density:
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 98 ppb a.i. 95% C.I.: 72-130 ppb a.i.
EC_{50}/IC_{50}: 240 ppb a.i. 95% C.I.: 210-280 ppb a.i.
Slope: 4.16 ± 0.436

Growth rates:
NOAEC: 146.3 ppb a.i.
LOAEC: 356.4 ppb a.i.
EC_{05}: could not be determined 95% C.I.: N/A
EC_{50}/IC_{50}: could not be determined 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 60 ppb a.i. 95% C.I.: 47-77 ppb a.i.
EC_{50}/IC_{50}: 180 ppb a.i. 95% C.I.: 160-200 ppb a.i.
Slope: 3.45 ± 0.239

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 were similar to the study authors'; biomass was the most sensitive endpoint.

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 180 ppb a.i..

Cell density:
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 98 ppb a.i. 95% C.I.: 72-130 ppb a.i.
EC_{50}/IC_{50}: 240 ppb a.i. 95% C.I.: 210-280 ppb a.i.
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Growth rates:
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EC_{50}/IC_{50}: 395.3 ppb a.i. 95% C.I.: 317.4-473.2 ppb a.i. (Study-author reported)
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: <23.5 ppb a.i.
LOAEC: 23.5 ppb a.i.
EC_{05}: 60 ppb a.i. 95% C.I.: 47-77 ppb a.i.
EC_{50}/IC_{50}: 180 ppb a.i. 95% C.I.: 160-200 ppb a.i.
Slope: 3.45±0.239
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:

prothioconazole acute algae N. pelliculosa cell density
File: pnpell.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	154512.639	30902.528	127.167
Within (Error)	22	5346.148	243.007	
Total	27	159858.787		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F REJECT Ho: All equal

prothioconazole acute algae N. pelliculosa cell density
File: pnpell.dat 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	pooled control	206.425	206.425		
2	23.5	170.150	170.150	3.800	*
3	56.6	182.825	182.825	2.472	
4	146.3	142.400	142.400	6.707	*
5	356.4	53.175	53.175	16.054	*
6	889.5	0.125	0.125	21.611	*

Bonferroni t table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

prothioconazole acute algae N. pelliculosa cell density
File: pnpell.dat 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	pooled control	8			
2	23.5	4	23.944	11.6	36.275
3	56.6	4	23.944	11.6	23.600
4	146.3	4	23.944	11.6	64.025
5	356.4	4	23.944	11.6	153.250
6	889.5	4	23.944	11.6	206.300

prothioconazole acute algae N. pelliculosa cell density
File: pnpell.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	pooled control	8	206.425	206.425	206.425
2	23.5	4	170.150	170.150	176.488
3	56.6	4	182.825	182.825	176.488

4	146.3	4	142.400	142.400	142.400
5	356.4	4	53.175	53.175	53.175
6	889.5	4	0.125	0.125	0.125

prothioconazole acute algae N. pelliculosa cell density
File: pnpell.dat 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
pooled control	206.425				
23.5	176.488	3.136	*	1.72	k= 1, v=22
56.6	176.488	3.136	*	1.80	k= 2, v=22
146.3	142.400	6.707	*	1.83	k= 3, v=22
356.4	53.175	16.054	*	1.84	k= 4, v=22
889.5	0.125	21.611	*	1.85	k= 5, v=22

s = 15.589
Note: df used for table values are approximate when v > 20.

prothioconazole acute algae N. pelliculosa cum biomass
File: pnpellcb.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	112696099.000	22539219.799	508.051
Within (Error)	22	976009.320	44364.060	
Total	27	113672108.320		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F REJECT Ho: All equal

prothioconazole acute algae N. pelliculosa cum biomass
File: pnpellcb.dat 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	pooled control	5341.800	5341.800		
2	23.5	4503.600	4503.600	6.499	*
3	56.6	4448.700	4448.700	6.924	*
4	146.3	3019.200	3019.200	18.007	*
5	356.4	884.100	884.100	34.560	*
6	889.5	-42.900	-42.900	41.747	*

Bonferroni t table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

prothioconazole acute algae N. pelliculosa cum biomass
File: pnpellcb.dat 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	pooled control	8			
2	23.5	4	323.527	6.1	838.200
3	56.6	4	323.527	6.1	893.100
4	146.3	4	323.527	6.1	2322.600
5	356.4	4	323.527	6.1	4457.700
6	889.5	4	323.527	6.1	5384.700

prothioconazole acute algae N. pelliculosa cum biomass
File: pnpellcb.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	pooled control	8	5341.800	5341.800	5341.800
2	23.5	4	4503.600	4503.600	4503.600
3	56.6	4	4448.700	4448.700	4448.700
4	146.3	4	3019.200	3019.200	3019.200
5	356.4	4	884.100	884.100	884.100
6	889.5	4	-42.900	-42.900	-42.900

prothioconazole acute algae N. pelliculosa cum biomass
File: pnpellcb.dat 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
pooled control	5341.800				
23.5	4503.600	6.499	*	1.72	k= 1, v=22
56.6	4448.700	6.924	*	1.80	k= 2, v=22
146.3	3019.200	18.007	*	1.83	k= 3, v=22
356.4	884.100	34.560	*	1.84	k= 4, v=22
889.5	-42.900	41.747	*	1.85	k= 5, v=22

s = 210.628
Note: df used for table values are approximate when v > 20.

prothioconazole acute algae N. pelliculosa growth rate
File: pnpellgr.dat Transform: NO TRANSFORMATION

KRUSKAL - WALLIS' ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	pooled control	5547.375	5547.375	189.000
2	23.5	5348.750	5348.750	64.000
3	56.6	5423.750	5423.750	75.000
4	146.3	5160.000	5160.000	42.000
5	356.4	4107.250	4107.250	26.000
6	889.5	-2218.500	-2218.500	10.000

Calculated H Value = 24.313 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

prothioconazole acute algae N. pelliculosa growth rate
File: pnpellgr.dat Transform: NO TRANSFORMATION

DUNN'S MULTIPLE COMPARISON - KRUSKAL - WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 4 2 3 1
6	889.5	-2218.500	-2218.500	\
5	356.4	4107.250	4107.250	. \
4	146.3	5160.000	5160.000	. . \
2	23.5	5348.750	5348.750	. . . \
3	56.6	5423.750	5423.750	. . . . \
1	pooled control	5547.375	5547.375	* * . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - several SE values used

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 Diatom (*Navicula pelliculosa*)

Kern, M.E. and Lam, C.V. 2004. Toxicity of JAU 6476 Technical to the Freshwater Diatom *Navicula pelliculosa*. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX085 (J6883401), and sponsored by Bayer CropScience, RTP, NC. Experimental start date April 22, 2002 and experimental termination date April 26, 2002. The final report issued March 12, 2004.

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, cultures of *Navicula pelliculosa* were exposed to Prothioconazole (JAU 6476 Technical; purity 97.5%) under static conditions at nominal concentrations of 0 (negative and solvent controls), 26.0, 64.0, 160.0, 400.0, and 1000.0 µg a.i./L. The 0-hour measured concentrations were <2.6 (< LOQ, controls), 23.5, 56.6, 146.3, 356.4, and 889.5 µg a.i./L, while day 4 concentrations were <2.6 (< LOQ, controls), <2.6, 8.3, 34.8, 206.2, and 720.7 µg a.i./L (0-72% of nominal). Mean measured concentrations were <2.6 (< LOQ, controls), 12.4, 32.4, 90.6, 281.3 and 805.1 µg a.i./L (half the LOQ was used as a day 4 concentration in the calculation of the mean in the lowest treatment level). The 0-hour measured concentrations were used for toxicity estimates by the EPA reviewer, but the EAD reviewer felt that using mean measured concentrations were a better estimate of exposure, as concentrations in all treatments declined significantly by day 4. The study was conducted following U.S. EPA Guideline 123-2 and was in compliance with U.S. EPA (40 CFR, Part 160). The 96-hour cell density percent inhibitions were 18, 11, 31, 74, and 100% in the 12.4, 32.4, 90.6, 281.3 and 805.1 µg a.i./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 16, 17, 43, 83, and 101% in the 12.4, 32.4, 90.6, 281.3 and 805.1 µg a.i./L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 4, 2, 7, 26, and 140% in the 12.4, 32.4, 90.6, 281.3 and 805.1 µg a.i./L treatment groups, respectively. The NOEC was <12.4 µg a.i./L, the lowest test concentration, for cell density and biomass, while the NOEC for growth rate was 32.4 µg a.i./L (mean measured concentrations). The 96-hour EC_{50}/IC_{50} (and corresponding 95% confidence intervals) for cell density, biomass (area under the growth curve) and growth rate were 174.4 µg a.i./L (144.1-206.4 µg a.i./L), 121.7 µg a.i./L (108.4-137.1 µg a.i./L) and 451.4 µg a.i./L (431.0-473.2 µg a.i./L), respectively, based on mean measured concentrations.

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type: Static

Cell density: (EAD-reviewer; mean measured concentrations)
NOEC: <12.4 µg a.i./L
LOEC: 32.4 µg a.i./L.
EC_{50}/IC_{50}: 174.4 µg a.i./L 95% C.I.: 144.1-206.4 µg a.i./L
Slope: N/A

Growth rates (EAD-reviewer; mean measured concentrations):
NOEC: 32.4 µg a.i./L
LOEC: 90.6 µg a.i./L
EC_{50}/IC_{50}: 451.4 µg a.i./L 95% C.I.: 431.0-473.2 µg a.i./L
Slope: N/A

Plant biomass (area under the growth curve) (EAD-reviewer; mean measured concentrations):
NOEC: <12.4 µg a.i./L
LOEC: 32.4 µg a.i./L
EC_{50}/IC_{50}: 121.7 µg a.i./L 95% C.I.: 108.4-137.1 µg a.i./L
Slope: N/A

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 verified the biomass and growth rate at 96 hours, based on the cell density and obtained identical results as the study authors.

3. The EPA reviewer followed the U.S. EPA policy of using the day 0 measured concentrations when levels decline below 70% of nominal. However, the EAD reviewer felt that using the mean measured concentrations to determine the EC_{50}/IC_{50} in this study was a better estimate of exposure, as concentrations after 4 days were significantly lower than on day 0 in all treatment vessels and using higher day 0 values would underestimate exposure and the differing results may affect the outcome of risk assessments. Mean measured concentrations were <2.6(LOQ, controls), 12.4, 32.4, 90.6, 281.3 and 805.1 µg a.i./L. Half the LOQ was used for the day 4 value in the calculation of the mean measured concentration for the nominal 26.0 µg a.i./L treatment, as the concentration measured was below the LOQ. Other algae studies with prothioconazole show

declines in concentrations in the lowest treatment levels after 4 days of exposure. The recovery in treatments with higher concentrations was often above 90% in other studies, which would indicate that the decline in concentration is not due to instability of the chemical. This difference is perhaps due to binding or absorption of the chemical or problems with detection, observed more at the lower concentrations. The concentrations used in this study are lower than in most of the other algae studies.

3. The EAD reviewer verified the NOEC for cell density, biomass and growth rate with an ANOVA using SigmaStat Statistical software. Solvent control and negative control were compared using t-tests and the data were pooled when no differences were detected between the two treatments. The EAD reviewer obtained the same results as the study authors and the EPA reviewer for cell density and biomass (NOEC <23.5 µg a.i./L, day 0; <12.4 µg a.i./L, mean measured). The assumption of normality was not met for growth rate and transformations were not successful. A non-parametric ANOVA was run and significant differences between the pooled controls and the three highest treatments were detected. The NOEC for growth rate is therefore 56.6 µg a.i./L (day 0) or 32.4 µg a.i./L mean measured). The study author reported the same value for the NOEC, but it was based on the determination of a lack of biologically significant differences at the two lowest treatment levels (significant statistical differences were obtained at all treatment levels). The EPA reviewer reported a NOEC of 146.3 µg a.i./L.

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

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

Cell density

One Way Analysis of VarianceMonday, October 31, 2005, 14:07:44

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.294)

Group Name	N	Missing	Mean	Std Dev	SEM
control	8	0	206.425	20.428	7.222
23.5 mg ai/L	4	0	170.150	12.391	6.195
56.6 mg ai/L	4	0	182.825	12.236	6.118
147.3 mg ai/L	4	0	142.400	15.960	7.980
356.4 mg ai/L	4	0	53.175	15.824	7.912
889.5 mg ai/L	4	0	0.125	0.0500	0.0250

Source of Variation	DF	SS	MS	F	P
Between Groups	5	154512.639	30902.528	127.167	<0.001
Residual	22	5346.147	243.007		
Total	27	159858.787			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatment

Comparison	Diff of Means	t	P	P<0.050
control vs. 889.5 mg ai/L	206.300	21.611	<0.001	Yes
control vs. 356.4 mg ai/L	153.250	16.054	<0.001	Yes
control vs. 147.3 mg ai/L	64.025	6.707	<0.001	Yes
control vs. 23.5 mg ai/L	36.275	3.800	0.005	Yes
control vs. 56.6 mg ai/L	23.600	2.472	0.108	No

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	12.4	32.4	90.6	281.3	805.1
Response	1	222.8	160.8	180	155.8	50.1	0.1
Response	2	180.5	186	200	122.5	39	0.2
Response	3	216	159.8	180.3	136.5	47.8	0.1

```
Response  4      213.5      174      171      154.8      75.8      0.1
Response  5      190.3
Response  6        232
Response  7        218
Response  8      178.3
-------------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole  Cell density Mean meas.
Test Start Date:    Test Ending Date:
Test Species: Navicula pelliculosa
Test Duration:        96 hours
DATA FILE: navdenpm.icp
OUTPUT FILE: navdenpm.i50
-------------------------------------------------------------------------
Conc.      Number      Concentration      Response      Std.       Pooled
 ID      Replicates       mg a.i./L        Means        Dev.   Response Means
-------------------------------------------------------------------------
  1          8             0.000        206.425     20.428    206.425
  2          4            12.400        170.150     12.391    176.488
  3          4            32.400        182.825     12.236    176.488
  4          4            90.600        142.400     15.960    142.400
  5          4           281.300         53.175     15.824     53.175
  6          4           805.100          0.125      0.050      0.125
-------------------------------------------------------------------------
The Linear Interpolation Estimate:   174.3552   Entered P Value: 50
-------------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean: 173.8998 Standard Deviation:    15.7530
Original Confidence Limits:   Lower:   144.1088 Upper:   206.3945
Expanded Confidence Limits:   Lower:   138.0595 Upper:   212.8023
Resampling time in Seconds:     0.00  Random_Seed: 2039632832
```

Biomass

One Way Analysis of Variance Monday, October 31, 2005, 14:08:53

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.188)

Group Name	N	Missing	Mean	Std Dev	SEM
control	8	0	5341.800	268.648	94.981
23.5 mg ai/L	4	0	4503.600	256.014	128.007
56.6 mg ai/L	4	0	4448.700	154.719	77.359
147.3 mg ai/L	4	0	3019.200	171.934	85.967
356.4 mg ai/L	4	0	884.100	194.181	97.091
889.5 mg ai/L	4	0	-42.900	13.695	6.848

Source of Variation	DF	SS	MS	F	P
Between Groups	5	112696098.994	22539219.799	508.051	<0.001
Residual	22	976009.320	44364.060		

Total	27	113672108.314

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatment

Comparison	Diff of Means	t	P	P<0.050
control vs. 889.5 mg ai/L	5384.700	41.747	<0.001	Yes
control vs. 356.4 mg ai/L	4457.700	34.560	<0.001	Yes
control vs. 147.3 mg ai/L	2322.600	18.007	<0.001	Yes
control vs. 56.6 mg ai/L	893.100	6.924	<0.001	Yes
control vs. 23.5 mg ai/L	838.200	6.499	<0.001	Yes

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	12.4	32.4	90.6	281.3	805.1
Response	1	5752.8	4586.4	4231.2	2923.2	817.2	-25.2
Response	2	5137.2	4776	4574.4	3080.4	688.8	-50.4
Response	3	5476.8	4164	4542	2842.8	880.8	-56.4
Response	4	5192.4	4488	4447.2	3230.4	1149.6	-39.6
Response	5	5509.2					
Response	6	5486.4					
Response	7	5287.2					
Response	8	4892.4					

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole Biomass Mean measured
Test Start Date: Test Ending Date:
Test Species: Navicula pelliculosa
Test Duration: 96 hours
DATA FILE: navbiom.icp
OUTPUT FILE: navbiom.i50

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	8	0.000	5341.800	268.648	5341.800
2	4	12.400	4503.600	256.014	4503.600
3	4	32.400	4448.700	154.719	4448.700
4	4	90.600	3019.200	171.934	3019.200
5	4	281.300	884.100	194.181	884.100
6	4	805.100	-42.900	13.695	0.000

The Linear Interpolation Estimate: 121.7090 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 122.3364 Standard Deviation: 7.6863
Original Confidence Limits: Lower: 108.4091 Upper: 137.1290

Expanded Confidence Limits: Lower: 105.7491 Upper: 140.2130
Resampling time in Seconds: 0.00 Random_Seed: 914796694

Growth Rate

RANKS Growth rate

One Way Analysis of Variance Monday, October 31, 2005, 14:12:49

Data source: Data 1 in Notebook

Normality Test: Failed (P = 0.008)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Monday, October 31, 2005, 14:12:49

Data source: Data 1 in Notebook

Group	N	Missing	Median		25%	75%
control	8	0	0.0559	0.0544	0.0562	
23.5 mg ai/L	4	0	0.0533	0.0529	0.0541	
56.6 mg ai/L	4	0	0.0541	0.0538	0.0547	
147.3 mg ai/L	4	0	0.0519	0.0506	0.0526	
356.4 mg ai/L	4	0	0.0405	0.0392	0.0429	
889.5 mg ai/L	4	0	-0.0240	-0.0240	-0.0204	

H = 24.313 with 5 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunn's Method) :

Comparison	Diff of Ranks	Q	P<0.05
889.5 mg ai/L vs control	21.125	4.194	Yes
356.4 mg ai/L vs control	17.125	3.400	Yes
147.3 mg ai/L vs control	13.125	2.606	Yes
23.5 mg ai/L vs control	7.625	1.514	No
56.6 mg ai/L vs control	4.875	0.968	Do Not Test

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	12.4	32.4	90.6	281.3	805.1
Response	1	0.05632	0.05292	0.05409	0.05259	0.04077	-0.02399
Response	2	0.05412	0.05444	0.05519	0.05008	0.03816	-0.01677
Response	3	0.05599	0.05285	0.05411	0.05121	0.04028	-0.02399
Response	4	0.05587	0.05374	0.05356	0.05252	0.04508	-0.02399
Response	5	0.05467					
Response	6	0.05674					
Response	7	0.05609					
Response	8	0.05399					

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole Growth rate Mean meas.
Test Start Date: Test Ending Date:
Test Species: Navicula pelliculosa
Test Duration: 96 hours
DATA FILE: navgropm.icp
OUTPUT FILE: navgropm.i50

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	8	0.000	0.055	0.001	0.055
2	4	12.400	0.053	0.001	0.054
3	4	32.400	0.054	0.001	0.054
4	4	90.600	0.052	0.001	0.052
5	4	281.300	0.041	0.003	0.041
6	4	805.100	-0.022	0.004	0.000

The Linear Interpolation Estimate: 451.3700 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 451.2339 Standard Deviation: 10.9178
Original Confidence Limits: Lower: 430.9977 Upper: 473.1665
Expanded Confidence Limits: Lower: 426.9232 Upper: 477.5258
Resampling time in Seconds: 0.00 Random_Seed: 2072874362