

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants

Lemna gibba

PMRA Submission #:2004-0844

EPA MRID#: 46246102

Data Requirement: PMRA Data Code: 9.8.6
EPA DP Barcode: D303495
OECD Data Point: IIIA 8.2.1
EPA MRID: 46246102
EPA Guideline: 123-2

Test material: JAU 6476 480 SC **Purity:** 42.1%
Common name: Prothioconazole formulation
Active Ingredient: Prothioconazole
Chemical name: IUPAC: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4,-triazole-3-thione
CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4,-triazole-3-thione
CAS No.: 178928-70-6
Synonyms: JAU6476

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Staff Scientist, Dynamac Corporation

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Date: 8/16/2005

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Date: 9/1/2005

Reference/Submission No.: 2004-0844

Company Code: BCZ

Active Code: PRB

Use Site Category: 7, 13, 14

EPA PC Code: 113961

Date Evaluation Completed:

CITATION: Kern, M.E., Banman, C.S., and Lam, C.V. 2003. Toxicity of JAU 6476 480 SC to Duckweed (*Lemna gibba* G3) Under Static-Renewal Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX189 (J6883703), and sponsored by Bayer CropScience, RTP, NC. Experimental start date February 7, 2003 and experimental termination date February 14, 2003. The final report issued December 16, 2003.



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Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX189 (J6883703), and sponsored by Bayer CropScience, RTP, NC. Experimental start date February 7, 2003 and experimental termination date February 14, 2003. The final report issued December 16, 2003.

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to the formulation JAU 6476 480 SC (42.1% Prothioconazole) at nominal concentrations of 0 (negative and formulation blank controls), 2.0, 6.7, 22.2, 74.1, 247, and 823 ppb a.i. under static renewal conditions. The measured concentrations were ≤ 0.98 (<LOD, negative and formulation blank controls), 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i.. The percent inhibitions for mean live frond numbers were -5, 1, 19, 62, 60, and 67% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control. The percent inhibitions for dry weights were -3, -6, 24, 53, 54, and 63% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control. The percent inhibitions for growth rates were 1, 3, 10, 38, 36, and 43% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for areas under the growth curve were -5, -2, 17, 51, 49, and 55% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control. The NOAEC for dry weight could not be determined (i.e., <2.3 ppb a.i.); the NOAEC for all other endpoints was 7.5 ppb a.i.. Frond number was the most sensitive endpoint tested with an EC_{50} of 110 ppb a.i..

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Acceptable.

Results Synopsis

Test Organism: *Lemna gibba* G3

Test Type: Static Renewal

Number of fronds:

NOAEC: 7.5 ppb a.i.

LOAEC: 28.8 ppb a.i.

EC_{05} : 0.64 ppb a.i.

95% C.I.: 0.088-4.6 ppb a.i.

EC_{50}/IC_{50} : 110 ppb a.i.

95% C.I.: 54-210 ppb a.i.

Slope: 0.740 ± 0.107

Growth rates (0-7 day):

NOAEC: 7.5 ppb a.i.

LOAEC: 28.8 ppb a.i.

EC_{05} : 1.6 ppb a.i.

95% C.I.: 0.21-12 ppb a.i.

EC_{50}/IC_{50} : >963 ppb a.i.

95% C.I.: N/A

Slope: 0.573 ± 0.0913

Plant biomass (area under the growth curve):

NOAEC: 7.5 ppb a.i.

LOAEC: 28.8 ppb a.i.

EC_{05} : 0.63 ppb a.i.

95% C.I.: 0.060-6.7 ppb a.i.

EC_{50}/IC_{50} : 240 ppb a.i.

95% C.I.: 120-490 ppb a.i.

Slope: 0.639 ± 0.107

Dry Weights:

NOAEC: 7.5 ppb a.i.

LOAEC: 28.8 ppb a.i.

EC_{05} : 0.48 ppb a.i.

95% C.I.: 0.062-3.7 ppb a.i.

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EC₅₀/IC₅₀: 130 ppb a.i. 95% C.I.: 66-250 ppb a.i.
Slope: 0.677±0.0967

Most Sensitive Endpoint: Frond number

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: U.S. Environmental Protection Agency, Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS 850.4400: Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II (1996). The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The dilution water characteristics were not reported.
2. The number of plants (3) was slightly less than the required 5 plants, however, there were 16 fronds per replicate.

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 test was conducted in accordance with 40 CFR Part 160.

A. MATERIALS:

1. Test Material Prothioconazole (JAU 6476 480 SC)

Description: White, milky liquid.

Lot No./Batch No. : 0030115

Purity: 42.1%

Stability of Compound

Under Test Conditions: The new test concentrations (days 0 and 3) were 112-130% of nominal concentrations and the old test concentrations (day 7) were 0-47% of nominal concentrations.

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

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

Storage conditions

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

2. Test organism:

Name: Duckweed, *Lemna gibba*

(EPA requires a vascular species: *Lemna gibba*.)

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Strain, if provided: G3

Source: Laboratory cultures (original supplier: Department of Horticulture Science, University of Minnesota, St. Paul, Minnesota).

Age of inoculum: 7 days old

Method of cultivation: 20X AAP Medium

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A range-finding study was conducted to determine the nominal test concentrations for the definitive test. The test concentrations were 0.30, 3.0, 30.0, 300, and 3000 ppb a.i.. The test concentrations were compared to a pooled control (dilution water control and formulation blank control). The percent inhibitions for frond counts were -5, -4, 30, 64, and 78% in the 0.30, 3.0, 30.0, 300, and 3000 ppb a.i. treatment groups, respectively. The percent inhibitions for dry weights were -1, -5, 26, 52, and 67% in the 0.30, 3.0, 30.0, 300, and 3000 ppb a.i. treatment groups, respectively.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	7 days	
culturing media and conditions: (same as test or not)	20X AAP Medium; same as test.	
health: (any toxicity observed)	The batch culture was in log phase growth.	
Test system static/static renewal/ renewal rate for static renewal:	Static Renewal Day 3	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Environmental chamber	
Duration of the test	7 days	<i>EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>

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Parameter	Details	Remarks
		Criteria
Test vessel material: (glass/polystyrene) size: fill volume:	Borosilicate glass crystallization dishes 650 mL (125 mm diameter and 65 mm height) 260 mL	Test vessels were covered with petri dish lids.
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP Medium 7.8-8.0 8.7-8.9 disodium EDTA NaHCO ₃	<i>EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Distilled water 7.5 Filter-sterilized (0.22 µm) and pH-adjusted with dilute hydrochloric acid N/A N/A N/A N/A N/A	The dilution water characteristics were not reported. <i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions	
Aeration or agitation	Not reported.	
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable.	

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Parameter	Details	Remarks
		Criteria
Number of replicates control: solvent control: treatments:	3 3 (formulation blank) 3	
Number of plants/replicate	Three plant with 16 fronds per replicate.	There were three plants for each treatment level. <i>EPA requires 5 plants.</i>
Number of fronds/plant	16 total fronds per replicate	<i>EPA requires 3 fronds per plant.</i>
Test concentrations nominal: measured:	0 (negative and formulation blank controls), 2.0, 6.7, 22.2, 74.1, 247, and 823 ppb a.i. ≤0.98 (<LOD, negative and formulation blank controls), 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i.	The mean measured test concentrations are based on day 0 and 3 new solutions. <i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; Days 0 (new solutions), 3 (new solutions), and 7 (old solutions).	
Test conditions temperature: photoperiod: light intensity and quality:	24.1-25.5°C continuous light 5.0 klux, cool-white fluorescent light	<i>EPA temperature: 25°C</i> <i>EPA photoperiod: continuous</i> <i>EPA light: 5.0 Klux (±15%)</i>
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

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Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds and dry weights.	
Measurement technique for frond number and other end points	Direct counts.	
Observation intervals	0, 3, 5, and 7 days.	
Other observations, if any	Area under the growth curve and growth rates were calculated.	
Indicate whether there was an exponential growth in the control	Yes, frond numbers in the dilution water (negative) and formulation blank controls on day 7 were approximately 15-16x frond numbers on day 0.	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The percent inhibitions for mean live frond numbers were -5, 1, 19, 62, 60, and 67% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control. The percent inhibitions for dry weights were -3, -6, 24, 53, 54, and 63% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control. The percent inhibitions for growth rates were 1, 3, 10, 38, 36, and 43% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for areas under the growth curve were -5, -2, 17, 51, 49, and 55% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control.

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Table 3: Effect of Prothioconazole formulation on frond number and dry weight of Duckweed, *Lemna gibba*

Treatment measured (nominal) concentrations ppb a.i.	Initial frond number/ test solution	Mean live frond number at				Mean dry weight (g) ^a	Mean Growth Rate ^b	Mean Area Under the Growth Curve ^a
		3 days	5 days	7 days	% inhibition at 7 days ^a			
Negative control (dilution water)	16	47	104	266	---	0.0337	0.01673	12076
Formulation blank control	16	47	92	233	--	0.0272	0.01595	10704
2.27 (2.0)	16	46	99	245	-5	0.0280	0.01622	11252
7.53 (6.7)	16	49	96	230	1	0.0289	0.01586	10940
28.8 (22.2)	16	44	81	189	19*	0.0207*	0.01469**	8908*
88.6 (74.1)	16	39	60	88	62*	0.0129*	0.01013**	5200*
288.9 (247)	16	39	61	94	60*	0.0125*	0.01050**	5412*
963.0 (823)	16	40	57	76	67*	0.0101*	0.00930**	4820*
Reference chemical (if used)	N/A							

^a The treatment groups were compared to the formulation blank control for day 7 frond counts, dry weights, and biomass.

^b The growth rate treatment groups were compared to the pooled control.

* Statistically different from the formulation blank control (Dunnett's one tailed test; p<0.05).

**Statistically different from the pooled control (Dunnett's one tailed test; p<0.05).

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Table 4: Statistical endpoint values.

Statistical Endpoint ^a	frond No.	dry weight	growth rate	area under the growth curve (biomass)
NOAEC or EC ₀₅ (ppb a.i.)	7.5	7.5	7.5	7.5
LOAEC (ppb a.i.)	28.8	28.8	28.8	28.8
EC ₅₀ (ppb a.i.) (95% C.I.)	66	961	>963	370
EC ₂₅ (ppb a.i.) (95% C.I.)	35	Not reported	83	32
Reference chemical NOAEC IC ₂₅ /EC ₂₅	Not applicable	Not applicable	Not applicable	Not applicable

^a Results are based on mean measured test concentrations.

B. REPORTED STATISTICS: The formulas for growth rate and area under the growth curve (biomass) are found on page 48. The growth data was analyzed using a t-test for the controls (formulation controls used for frond count, biomass, and dry weight comparisons, and pooled controls used for growth rate 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 using SAS version 8 computer programs. The NOAEC and LOAEC were determined from analyzed data. Nonparametric analyses were conducted for the dry weight data. Parametric analyses were conducted for the frond counts, biomass, and growth rate data. All statistical calculations were performed using the mean measured concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Day 7 frond count, biomass, growth rate, and dry weight data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). The NOAEC and LOAEC for these endpoints were determined using ANOVA, followed by William's multiple comparison test. With the exception of dry weight, the solvent control was compared to the nutrient control using a Student's t-test and, upon finding no significant differences, the treatment groups were compared to the pooled control group; for dry weight, there was a difference between the two control groups, so the treatment groups were compared to the solvent control. The analyses described above were conducted using TOXSTAT statistical software. The EC₀₅ and EC₅₀ values were determined using the Probit method via Nuthatch statistical software.

Number of fronds:

NOAEC: 7.5 ppb a.i.

LOAEC: 28.8 ppb a.i.

EC₀₅: 0.64 ppb a.i.

95% C.I.: 0.088-4.6 ppb a.i.

EC₅₀/IC₅₀: 110 ppb a.i.

95% C.I.: 54-210 ppb a.i.

Slope: 0.740±0.107

Growth rates (0-7 day):

NOAEC: 7.5 ppb a.i.

LOAEC: 28.8 ppb a.i.

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EC₀₅: 1.6 ppb a.i. 95% C.I.: 0.21-12 ppb a.i.
EC₅₀/IC₅₀: >963 ppb a.i. 95% C.I.: N/A
Slope: 0.573±0.0913

Plant biomass (area under the growth curve):

NOAEC: 7.5 ppb a.i.
LOAEC: 28.8 ppb a.i.
EC₀₅: 0.63 ppb a.i. 95% C.I.: 0.060-6.7 ppb a.i.
EC₅₀/IC₅₀: 240 ppb a.i. 95% C.I.: 120-490 ppb a.i.
Slope: 0.639±0.107

Dry Weights:

NOAEC: 7.5 ppb a.i.
LOAEC: 28.8 ppb a.i.
EC₀₅: 0.48 ppb a.i. 95% C.I.: 0.062-3.7 ppb a.i.
EC₅₀/IC₅₀: 130 ppb a.i. 95% C.I.: 66-250 ppb a.i.
Slope: 0.677±0.0967

Most Sensitive Endpoint: Frond number

D. STUDY DEFICIENCIES:

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

E. REVIEWER'S COMMENTS:

The reviewer's statistical verification provided similar results as the study authors'. Both concluded that frond count (standing crop) was the most sensitive endpoint, based on the EC₅₀ value (110 ppb a.i.). The reviewer's toxicity estimates are provided in the Executive Summary and Conclusions sections because they were associated with 95% confidence intervals and slope values.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Acceptable. Frond number was the most sensitive endpoint tested with an EC₅₀ of 110 ppb a.i..

Number of fronds:

NOAEC: 7.5 ppb a.i.
LOAEC: 28.8 ppb a.i.
EC₀₅: 0.64 ppb a.i. 95% C.I.: 0.088-4.6 ppb a.i.
EC₅₀/IC₅₀: 110 ppb a.i. 95% C.I.: 54-210 ppb a.i.
Slope: 0.740±0.107

Growth rates (0-7 day):

NOAEC: 7.5 ppb a.i.
LOAEC: 28.8 ppb a.i.
EC₀₅: 1.6 ppb a.i. 95% C.I.: 0.21-12 ppb a.i.
EC₅₀/IC₅₀: >963 ppb a.i. 95% C.I.: N/A

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Slope: 0.573 ± 0.0913

Plant biomass (area under the growth curve):

NOAEC: 7.5 ppb a.i.

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EC₅₀/IC₅₀: 130 ppb a.i. 95% C.I.: 66-250 ppb a.i.

Slope: 0.677 ± 0.0967

Most Sensitive Endpoint: Frond number

III. REFERENCES:

American Public Health Association (APHA). 1998. **Standard Methods for the Examination of Water and Wastewater, 20th edition**. Washington, D.C.

American Society for Testing and Materials (ASTM). 1991. Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3. ASTM Standard E1415. Philadelphia, PA.

Bruce, R.D. and D.J. Versteeg, 1992. "A Statistical Procedure for Modeling Continuous Data" Environmental Toxicology and Chemistry, Volume 11, pgs 1485-1494.

Microsoft Excel, 1997. Release 97 for Windows. Microsoft Corporation.

SAS Institute. 1999. PC-SAS version 8. Cary, NC.

Schneider, J. 2001. Physical and Chemical Properties of JAU 6476. Bayer AG, Leverkusen, Germany. Laboratory Project ID: 14 01200950.

Stein, J.R. (Ed.). 1973. **Handbook of Phycological Methods: Culture Methods and Growth Measurements**. Cambridge University Press., Cambridge, England.

USEPA, 1982. Pesticide Assessment Guidelines, Subdivision J-Hazard Evaluation: Nontarget Plants. EPA 540/9-82-020. Office of Pesticide Programs, Washington, D.C. 55 pp.

USEPA. 1985. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA 600/4-89/001. Office of Research and Development, Cincinnati, OH.

USEPA, 1986. Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants-Tiers 1 and 2. EPA-540/9-86-134. Office of Pesticide Programs, Washington, D.C.

USEPA, 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal

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Register, Vol. 54, No. 158: 34067-34074.

USEPA, 1994. Pesticide Reregistration Rejection Rate Analysis. Ecological Effects. EPA 738-R-94-035: p 160.

USEPA, 1996. Series 850-Ecological Effects test Guidelines (*draft*). OPPTS 850.4400: Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond count

File: 6102fc

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	129220.500	21536.750	82.445
Within (Error)	17	4440.833	261.225	
Total	23	133661.333		

Critical F value = 2.70 (0.05,6,17)

Since F > Critical F REJECT Ho:All groups equal

frond count

File: 6102fc

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	249.833	249.833		
2	2.27	244.667	244.667	0.452	
3	7.53	230.000	230.000	1.735	
4	28.8	188.667	188.667	5.352	*
5	88.6	88.000	88.000	14.160	*
6	289	94.000	94.000	13.635	*
7	963	76.333	76.333	15.181	*

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

frond count

File: 6102fc

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	2.27	3	30.343	12.1	5.167
3	7.53	3	30.343	12.1	19.833
4	28.8	3	30.343	12.1	61.167
5	88.6	3	30.343	12.1	161.833
6	289	3	30.343	12.1	155.833
7	963	3	30.343	12.1	173.500

frond count

File: 6102fc

Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model)			TABLE 1 OF 2		
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	249.833	249.833	249.833
2	2.27	3	244.667	244.667	244.667
3	7.53	3	230.000	230.000	230.000
4	28.8	3	188.667	188.667	188.667
5	88.6	3	88.000	88.000	91.000
6	289	3	94.000	94.000	91.000
7	963	3	76.333	76.333	76.333

frond count
File: 6102fc

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	249.833				
2.27	244.667	0.452		1.74	k= 1, v=17
7.53	230.000	1.735		1.82	k= 2, v=17
28.8	188.667	5.352	*	1.85	k= 3, v=17
88.6	91.000	13.898	*	1.87	k= 4, v=17
289	91.000	13.898	*	1.87	k= 5, v=17
963	76.333	15.181	*	1.88	k= 6, v=17

s = 16.162

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.64	0.088	4.6	0.41	0.14
EC10	2.0	0.38	10.	0.35	0.19
EC25	13.	4.2	41.	0.24	0.32
EC50	1.1E+02	54.	2.1E+02	0.14	0.51

Slope = 0.740 Std.Err. = 0.107

!!!Poor fit: p < 0.001 based on DF= 4.00 17.0

6102FC : frond count

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	250.	259.	-8.89	100.	0.00
2.27	3.00	245.	231.	13.9	89.2	10.8
7.53	3.00	230.	208.	22.3	80.3	19.7
28.8	3.00	189.	171.	17.2	66.3	33.7
88.6	3.00	88.0	135.	-47.4	52.3	47.7

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289.	3.00	94.0	96.8	-2.78	37.4	62.6
963.	3.00	76.3	62.0	14.4	24.0	76.0

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

biomass

File: 6102b

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	192452232.000	32075372.000	49.745
Within (Error)	17	10961592.000	644799.529	
Total	23	203413824.000		

Critical F value = 2.70 (0.05,6,17)

Since F > Critical F REJECT Ho:All groups equal

biomass

File: 6102b

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	11390.000	11390.000		
2	2.27	11252.000	11252.000	0.243	
3	7.53	10940.000	10940.000	0.793	
4	28.8	8908.000	8908.000	4.371	*
5	88.6	5200.000	5200.000	10.902	*
6	289	5412.000	5412.000	10.528	*
7	963	4820.000	4820.000	11.571	*

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

biomass

File: 6102b

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	2.27	3	1507.516	13.2	138.000
3	7.53	3	1507.516	13.2	450.000
4	28.8	3	1507.516	13.2	2482.000
5	88.6	3	1507.516	13.2	6190.000
6	289	3	1507.516	13.2	5978.000
7	963	3	1507.516	13.2	6570.000

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biomass
File: 6102b 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	11390.000	11390.000	11390.000
2	2.27	3	11252.000	11252.000	11252.000
3	7.53	3	10940.000	10940.000	10940.000
4	28.8	3	8908.000	8908.000	8908.000
5	88.6	3	5200.000	5200.000	5306.000
6	289	3	5412.000	5412.000	5306.000
7	963	3	4820.000	4820.000	4820.000

biomass
File: 6102b 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	11390.000				
2.27	11252.000	0.243		1.74	k= 1, v=17
7.53	10940.000	0.793		1.82	k= 2, v=17
28.8	8908.000	4.371	*	1.85	k= 3, v=17
88.6	5306.000	10.715	*	1.87	k= 4, v=17
289	5306.000	10.715	*	1.87	k= 5, v=17
963	4820.000	11.571	*	1.88	k= 6, v=17

s = 802.994

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	0.63	0.060 6.7	0.49	0.094
EC10	2.4	0.34 16.	0.40	0.15
EC25	21.	6.0 73.	0.26	0.29
EC50	2.4E+02	1.2E+02 4.9E+02	0.15	0.49

Slope = 0.639 Std.Err. = 0.107

!!!Poor fit: p < 0.001 based on DF= 4.00 17.0

6102B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
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0.00	6.00	1.14e+04	1.18e+04	-377.	100.	0.00
2.27	3.00	1.13e+04	1.06e+04	641.	90.2	9.83
7.53	3.00	1.09e+04	9.78e+03	1.16e+03	83.1	16.9
28.8	3.00	8.91e+03	8.49e+03	421.	72.1	27.9
88.6	3.00	5.20e+03	7.16e+03	-1.96e+03	60.8	39.2
289.	3.00	5.41e+03	5.64e+03	-223.	47.9	52.1
963.	3.00	4.82e+03	4.11e+03	708.	34.9	65.1

!!!Warning: EC5 not bracketed by doses evaluated.

growth rate

File: 6102g

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	20480.280	3413.380	126.276
Within (Error)	17	459.533	27.031	
Total	23	20939.813		

Critical F value = 2.70 (0.05,6,17)

Since F > Critical F REJECT Ho:All groups equal

growth rate

File: 6102g

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	163.370	163.370		
2	2.27	162.183	162.183	0.323	
3	7.53	158.600	158.600	1.297	
4	28.8	146.857	146.857	4.492	*
5	88.6	101.263	101.263	16.894	*
6	289	105.033	105.033	15.868	*
7	963	92.957	92.957	19.153	*

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

growth rate

File: 6102g

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	2.27	3	9.761	6.0	1.187
3	7.53	3	9.761	6.0	4.770
4	28.8	3	9.761	6.0	16.513

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5	88.6	3	9.761	6.0	62.107
6	289	3	9.761	6.0	58.337
7	963	3	9.761	6.0	70.413

growth rate

File: 6102g

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	163.370	163.370	163.370
2	2.27	3	162.183	162.183	162.183
3	7.53	3	158.600	158.600	158.600
4	28.8	3	146.857	146.857	146.857
5	88.6	3	101.263	101.263	103.148
6	289	3	105.033	105.033	103.148
7	963	3	92.957	92.957	92.957

growth rate

File: 6102g

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	163.370				
2.27	162.183	0.323		1.74	k= 1, v=17
7.53	158.600	1.297		1.82	k= 2, v=17
28.8	146.857	4.492	*	1.85	k= 3, v=17
88.6	103.148	16.381	*	1.87	k= 4, v=17
289	103.148	16.381	*	1.87	k= 5, v=17
963	92.957	19.153	*	1.88	k= 6, v=17

s = 5.199

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.6	0.21	12.	0.42	0.13
EC10	6.8	1.4	33.	0.33	0.21
EC25	78.	32.	1.9E+02	0.19	0.41
EC50	1.2E+03	6.0E+02	2.3E+03	0.14	0.52

Slope = 0.573 Std.Err. = 0.0913

!!!Poor fit: p < 0.001 based on DF= 4.00 17.0

6102G : growth rate

Observed vs. Predicted Treatment Group Means

**Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants
*Lemna gibba***

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Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	163.	167.	-3.56	100.	0.00
2.27	3.00	162.	157.	5.28	94.0	6.01
7.53	3.00	159.	149.	9.13	89.5	10.5
28.8	3.00	147.	137.	9.69	82.2	17.8
88.6	3.00	101.	123.	-22.2	74.0	26.0
289.	3.00	105.	106.	-1.14	63.6	36.4
963.	3.00	93.0	86.7	6.27	51.9	48.1

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

dry weight

Leman JAU6475 480 SC dry weight

File: lemn102 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	120442.571	20073.762	70.493
Within (Error)	14	3986.667	284.762	
Total	20	124429.238		

Critical F value = 2.85 (0.05,6,14)

Since F > Critical F REJECT Ho: All equal

Leman JAU6475 480 SC dry weight

File: lemn102 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	form blank	272.333	272.333		
2	2.27	279.667	279.667	-0.532	
3	7.53	289.333	289.333	-1.234	
4	28.8	207.333	207.333	4.718	*
5	88.6	129.333	129.333	10.379	*
6	289	124.667	124.667	10.717	*
7	963	100.667	100.667	12.459	*

Bonferroni t table value = 2.72 (1 Tailed Value, P=0.05, df=14,6)

Leman JAU6475 480 SC dry weight

File: lemn102 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	form blank	3			

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2	2.27	3	37.447	13.8	-7.333
3	7.53	3	37.447	13.8	-17.000
4	28.8	3	37.447	13.8	65.000
5	88.6	3	37.447	13.8	143.000
6	289	3	37.447	13.8	147.667
7	963	3	37.447	13.8	171.667

Leman JAU6475 480 SC dry weight
File: lemn102 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	form blank	3	272.333	272.333	280.444
2	2.27	3	279.667	279.667	280.444
3	7.53	3	289.333	289.333	280.444
4	28.8	3	207.333	207.333	207.333
5	88.6	3	129.333	129.333	129.333
6	289	3	124.667	124.667	124.667
7	963	3	100.667	100.667	100.667

Leman JAU6475 480 SC dry weight
File: lemn102 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
form blank	280.444				
2.27	280.444	0.589		1.76	k= 1, v=14
7.53	280.444	0.589		1.85	k= 2, v=14
28.8	207.333	4.718	*	1.88	k= 3, v=14
88.6	129.333	10.379	*	1.89	k= 4, v=14
289	124.667	10.717	*	1.90	k= 5, v=14
963	100.667	12.459	*	1.91	k= 6, v=14

s = 16.875

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.48	0.062	3.7	0.43	0.13
EC10	1.7	0.30	9.0	0.35	0.18
EC25	13.	4.2	41.	0.24	0.32
EC50	1.3E+02	66.	2.5E+02	0.14	0.51

Slope = 0.677 Std.Err. = 0.0967

!!!Poor fit: p = 0.0045 based on DF= 4.0 17.

6102DW : dry weight

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants***Lemna gibba***

PMRA Submission #:2004-0844

EPA MRID#: 46246102

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	3.05	3.12	-0.0728	100.	0.00
2.27	3.00	2.80	2.75	0.0434	88.3	11.7
7.53	3.00	2.89	2.49	0.403	79.8	20.2
28.8	3.00	2.07	2.09	-0.0181	67.0	33.0
88.6	3.00	1.29	1.70	-0.404	54.4	45.6
289.	3.00	1.25	1.27	-0.0210	40.6	59.4
963.	3.00	1.01	0.865	0.142	27.7	72.3

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA

Date: September 1, 2005

PMRA Submission Number: 2004-0844

Study Type: Acute Toxicity to Non-Target Plants- Laboratory Studies with the End-Use Product

Kern, M.E., Banman, C.S., and Lam, C.V. 2003. Toxicity of JAU 6476 480 SC to Duckweed (*Lemna gibba* G3) Under Static-Renewal Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX189 (J6883703), and sponsored by Bayer CropScience, RTP, NC. Experimental start date February 7, 2003 and experimental termination date February 14, 2003. The final report issued December 16, 2003.

PMRA DATA CODE: 9.8.6

EPA DP Barcode: D303488

OECD Data Point: IIA 8.6.1

EPA MRID: 46246102

EPA Guideline: 123-2

Reviewing Agency: US EPA

EAD Executive Summary:

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to the formulation JAU 6476 480 SC (42.1% prothioconazole) at nominal concentrations of 0 (negative and formulation blank controls), 2.0, 6.7, 22.2, 74.1, 247, and 823 µg a.i./L under static renewal conditions. The measured concentrations were ≤0.98 (<LOD, negative and formulation blank controls), 2.3, 7.5, 28.8, 88.6, 289, and 963 µg a.i./L. The percent inhibitions for mean live frond numbers were -5, 1, 19, 62, 60, and 67% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 µg a.i./L treatment groups, respectively, compared to the formulation blank control. The percent inhibitions for dry weights were -3, -6, 24, 53, 54, and 63% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 µg a.i./L treatment groups, respectively, compared to the formulation blank control. The percent inhibitions for growth rates were 1, 3, 10, 38, 36, and 43% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 µg a.i./L treatment groups, respectively, compared to the pooled control. The percent inhibitions for biomass (area under the growth curve) were -5, -2, 17, 51, 49, and 55% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 µg a.i./L treatment groups, respectively, compared to the formulation blank control. The NOEC for all endpoints was 7.5 µg a.i./L. The EC₅₀ (95% confidence intervals) values determined by the EAD reviewer were 71.1 (66.6-74.4), 80.2 (72.8-101.9), >963, and 85.6 (79.9-406.9) µg a.i./L for frond numbers, dry

weight, growth rate and biomass, respectively.

Results Synopsis, as determined by EAD reviewer

Test Organism: *Lemna gibba* G3

Test Type: Static Renewal

Number of fronds:

NOEC: 7.5 µg a.i./L

LOEC: 28.8 µg a.i./L

EC₀₅: 9.0 µg a.i./L 95% C.I.: 4.6-13.8 µg a.i./L

EC₅₀/IC₅₀: 71.1 µg a.i./L. 95% C.I.: 66.6-74.4 µg a.i./L

Growth rates (0-7 day):

NOEC: 7.5 µg a.i./L

LOEC: 28.8 µg a.i./L

EC₀₅: 13.7 µg a.i./L 95% C.I.: 6.4-19.9 µg a.i./L

EC₅₀/IC₅₀: >963 µg a.i./L 95% C.I.: N/A

Plant biomass (area under the growth curve):

NOEC: 7.5 µg a.i./L

LOEC: 28.8 µg a.i./L

EC₀₅: 12.9 µg a.i./L 95% C.I.: 4.7-14.3 µg a.i./L

EC₅₀/IC₅₀: 85.6 µg a.i./L 95% C.I.: 79.9-406.9 µg a.i./L

Dry Weights:

NOEC: 7.5 µg a.i./L

LOEC: 28.8 µg a.i./L

EC₀₅: 11.6 µg a.i./L 95% C.I.: 8.6-12.0 µg a.i./L

EC₅₀/IC₅₀: 80.2 µg a.i./L 95% C.I.: 72.8-101.9 µg a.i./L

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) available from the PMRA Chemistry review.

2. The OECD Guideline requires that the doubling time in the control must be less than 2.5 days

(60 hours). Based on the formula for the doubling time (T_d) of $T_d = \ln 2/\mu$, where μ is the average specific growth rate. Based on average specific growth rates for the controls (0.01673 for the negative control, 0.01595 for the formulation blank and 0.01634 for the pooled controls), the doubling time is 41-4-43.5 hours, satisfying the validity criteria for the study.

3. The area under the growth curve and the growth rate numbers were verified by the EAD reviewer. The reviewer obtained values identical to those reported by the study author.

4. Data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances), for frond numbers, growth rate and biomass (area under the growth curve) so the NOEC and LOEC were determined using this test followed by Dunnett's multiple comparison test. These analyses were conducted using SigmaStat statistical software. Controls were pooled when a t-test showed no significant differences between the negative control and the formulation blank.

Identical results as those of the study author and the EPA reviewer were obtained for frond numbers,

The assumption of homogeneity of variances was not met for dry weight data. A Kruskal-Wallis One Way ANOVA on Ranks did not indicate any significant differences except for the highest treatment level. The lack of significant differences is likely due to the low power of the non-parametric test and to the low sample size. After inspecting the data, it is clear that the first two treatments are adversely affected and therefore not different from the formulation blank, as the average weight is higher than that of the formulation blank. However, there is no overlap between the mean and 95% confidence limits of the formulation blank and the 28.8 $\mu\text{g a.i./L}$ and higher treatment levels. In addition, similar differences in effects between treatment levels were observed for the three other endpoints. Therefore, the EAD reviewer agrees with the study author that the NOEC for dry weight is 7.5 $\mu\text{g a.i./L}$.

The EC_{xx} values were calculated using a linear interpolation method. (Norberg-King, T. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). USEPA, Duluth, MN). Mean measured concentrations of the mixture were used for all toxicity determinations. The EC_{xx} values calculated by the EAD reviewer will be used by the PMRA, as opposed to those of the EPA reviewer.

Study Acceptability: This toxicity study is scientifically sound and satisfies the data requirements for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Acceptable.

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants
Lemna gibba

PMRA Submission #:2004-0844

EPA MRID#: 46246102

Statistical analyses of the EAD reviewer:

FronD numbers

Conc. ID	1	2	3	4	5	6	7
Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
Response 1	220	258	239	187	97	98	79
Response 2	234	256	216	194	88	80	72
Response 3	246	220	235	185	79	104	78

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole formulation FROND
Test Start Date: Test Ending Date:
Test Species: Lemna gibba
Test Duration: 168 hours
DATA FILE: frond.icp
OUTPUT FILE: frond.i05

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	233.333	13.013	239.000
2	3	2.270	244.667	21.385	239.000
3	3	7.530	230.000	12.288	230.000
4	3	28.800	188.667	4.726	188.667
5	3	88.600	88.000	9.000	91.000
6	3	289.000	94.000	12.490	91.000
7	3	963.000	76.333	3.786	76.333

The Linear Interpolation Estimate: 9.0481 Entered P Value: 5

Number of Resamplings: 80
The Bootstrap Estimates Mean: 9.5787 Standard Deviation: 2.9054
Original Confidence Limits: Lower: 4.6113 Upper: 13.8053
Expanded Confidence Limits: Lower: -0.2690 Upper: 19.0382
Resampling time in Seconds: 0.00 Random_Seed: 507983743

Conc. ID	1	2	3	4	5	6	7
Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
Response 1	220	258	239	187	97	98	79
Response 2	234	256	216	194	88	80	72
Response 3	246	220	235	185	79	104	78

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole formulation FROND
Test Start Date: Test Ending Date:
Test Species: Lemna gibba
Test Duration: 168 hours
DATA FILE: frond.icp
OUTPUT FILE: frond.i50

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants
Lemna gibba

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Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	233.333	13.013	239.000
2	3	2.270	244.667	21.385	239.000
3	3	7.530	230.000	12.288	230.000
4	3	28.800	188.667	4.726	188.667
5	3	88.600	88.000	9.000	91.000
6	3	289.000	94.000	12.490	91.000
7	3	963.000	76.333	3.786	76.333

The Linear Interpolation Estimate: 71.1498 Entered P Value: 50

Number of Resamplings: 80

The Bootstrap Estimates Mean: 70.6383 Standard Deviation: 2.2204

Original Confidence Limits: Lower: 66.5735 Upper: 74.3826

Expanded Confidence Limits: Lower: 61.5395 Upper: 77.9386

Resampling time in Seconds: 0.00 Random_Seed: 868711375

Growth rates

Conc. ID	1	2	3	4	5	6	7
----------	---	---	---	---	---	---	---

Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
--------------	---	------	------	------	------	-----	-----

Response 1 0.0162660.016550.0160940.0146340.0107270.0107880.009505

Response 2 0.01710.0165040.0154920.0148530.0101470.009580.008953

Response 3 0.016820.0156010.0159940.014570.0095050.0111420.009429

Response 4 0.015601

Response 5 0.015969

Response 6 0.016266

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Prothioconazole formulation GROWTH RATE

Test Start Date: Test Ending Date:

Test Species: Lemna gibba

Test Duration: 168 hours

DATA FILE: growth.icp

OUTPUT FILE: growth.i05

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.016	0.001	0.016
2	3	2.270	0.016	0.001	0.016
3	3	7.530	0.016	0.000	0.016
4	3	28.800	0.015	0.000	0.015
5	3	88.600	0.010	0.001	0.010
6	3	289.000	0.011	0.001	0.010
7	3	963.000	0.009	0.000	0.009

The Linear Interpolation Estimate: 13.6855 Entered P Value: 5

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants***Lemna gibba***

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EPA MRID#: 46246102

Number of Resamplings: 80
The Bootstrap Estimates Mean: 13.4843 Standard Deviation: 4.1568
Original Confidence Limits: Lower: 6.3725 Upper: 19.8516
Expanded Confidence Limits: Lower: 4.1786 Upper: 21.7014
Resampling time in Seconds: 0.00 Random_Seed: 28209560

Conc. ID	1	2	3	4	5	6	7
----------	---	---	---	---	---	---	---

Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
--------------	---	------	------	------	------	-----	-----

Response 1	0.0162660	0.016550	0.0160940	0.0146340	0.0107270	0.0107880	0.009505
Response 2	0.01710	0.0165040	0.0154920	0.0148530	0.0101470	0.009580	0.008953
Response 3	0.016820	0.0156010	0.0159940	0.014570	0.0095050	0.0111420	0.009429
Response 4	0.015601						
Response 5	0.015969						
Response 6	0.016266						

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Prothioconazole formulation GROWTH RATE

Test Start Date: Test Ending Date:

Test Species: *Lemna gibba*

Test Duration: 168 hours

DATA FILE: growth.icp

OUTPUT FILE: growth.i50

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.016	0.001	0.016
2	3	2.270	0.016	0.001	0.016
3	3	7.530	0.016	0.000	0.016
4	3	28.800	0.015	0.000	0.015
5	3	88.600	0.010	0.001	0.010
6	3	289.000	0.011	0.001	0.010
7	3	963.000	0.009	0.000	0.009

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 50% of the control response mean.

Biomass (Area under the growth curve)

Conc. ID	1	2	3	4	5	6	7
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Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
--------------	---	------	------	------	------	-----	-----

Response 1	101881191611604	9048	5700	6108	4536		
Response 2	106441186810056	8724	4800	4512	4872		
Response 3	11280	997211160	8952	5100	5616	5052	

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Prothioconazole formulation BIOMASS

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants***Lemna gibba***

PMRA Submission #:2004-0844

EPA MRID#: 46246102

Test Start Date: Test Ending Date:

Test Species: *Lemna gibba*

Test Duration: 168 hours

DATA FILE: biomass.icp

OUTPUT FILE: biomass.i05

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	10704.000	548.467	10978.000
2	3	2.270	11252.000	1108.772	10978.000
3	3	7.530	10940.000	797.105	10940.000
4	3	28.800	8908.000	166.421	8908.000
5	3	88.600	5200.000	458.258	5306.000
6	3	289.000	5412.000	817.322	5306.000
7	3	963.000	4820.000	261.901	4820.000

The Linear Interpolation Estimate: 12.8779 Entered P Value: 5

Number of Resamplings: 80

The Bootstrap Estimates Mean: 11.5320 Standard Deviation: 2.8958

Original Confidence Limits: Lower: 4.6693 Upper: 14.2986

Expanded Confidence Limits: Lower: -4.3601 Upper: 15.8614

Resampling time in Seconds: 0.00 Random_Seed: 1252149784

Conc. ID	1	2	3	4	5	6	7
Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
Response 1	101881191611604	9048	5700	6108	4536		
Response 2	106441186810056	8724	4800	4512	4872		
Response 3	11280	997211160	8952	5100	5616	5052	

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Prothioconazole formulation BIOMASS

Test Start Date: Test Ending Date:

Test Species: *Lemna gibba*

Test Duration: 168 hours

DATA FILE: biomass.icp

OUTPUT FILE: biomass.i50

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	10704.000	548.467	10978.000
2	3	2.270	11252.000	1108.772	10978.000
3	3	7.530	10940.000	797.105	10940.000
4	3	28.800	8908.000	166.421	8908.000
5	3	88.600	5200.000	458.258	5306.000
6	3	289.000	5412.000	817.322	5306.000
7	3	963.000	4820.000	261.901	4820.000

The Linear Interpolation Estimate: 85.5619 Entered P Value: 50

Number of Resamplings: 80

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants
Lemna gibba

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The Bootstrap Estimates Mean: 132.9526 Standard Deviation: 109.8805
Original Confidence Limits: Lower: 79.9472 Upper: 406.8978
Expanded Confidence Limits: Lower: 73.7711 Upper: 760.3674
Resampling time in Seconds: 0.00 Random_Seed: 1711077768

Dry weight

Weight on RANKS

One Way Analysis of Variance Wednesday, August 31, 2005, 10:31:15

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.081)

Equal Variance Test: Failed (P = 0.011)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, August 31, 2005, 10:31:15

Data source: Data 1 in Notebook

Group	N	Missing	Median	25%	75%
form. blank	3	0	0.0269	0.0269	0.0277
2.27 ug ai/L	3	0	0.0278	0.0265	0.0295
7.53 ug a.i./L	3	0	0.0294	0.0265	0.0313
28.8 ug a.i./L	3	0	0.0208	0.0204	0.0211
88.6 ug a.i./L	3	0	0.0127	0.0118	0.0141
289 ug a.i./L	3	0	0.0126	0.0114	0.0135
963 ug a.i./L	3	0	0.0101	0.00965	0.0105

H = 18.003 with 6 degrees of freedom. (P = 0.006)

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 = 0.006)

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
963 ug a.i./L vs form. blank	14.333	2.829	Yes
289 ug a.i./L vs form. blank	10.333	2.040	No
88.6 ug a.i./L vs form. blank	9.333	1.842	Do Not Test
28.8 ug a.i./L vs form. blank	5.333	1.053	Do Not Test
7.53 ug a.i./L vs form. blank	1.333	0.263	Do Not Test

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2.27 ug ai/L vs form. blank 0.667 0.132 Do Not Test

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

Weight

One Way Analysis of Variance Wednesday, August 31, 2005, 15:54:17

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.081)

Equal Variance Test: Failed (P = 0.011)

Group Name	N	Missing	Mean	Std Dev	SEM
form. blank	3	0	0.0272	0.000577	0.000333
2.27 ug ai/L	3	0	0.0280	0.00206	0.00119
7.53 ug a.i./L	3	0	0.0289	0.00323	0.00186
28.8 ug a.i./L	3	0	0.0207	0.000503	0.000291
88.6 ug a.i./L	3	0	0.0129	0.00156	0.000902
289 ug a.i./L	3	0	0.0125	0.00140	0.000811
963 ug a.i./L	3	0	0.0101	0.000551	0.000318

Source of Variation	DF	SS	MS	F	P
Between Groups	6	0.00120	0.000201	70.493	<0.001
Residual	14	0.0000399	0.00000285		
Total	20	0.00124			

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
form. blank vs. 963 ug a.i./L		0.0172	12.459	<0.001 Yes
form. blank vs. 289 ug a.i./L		0.0148	10.717	<0.001 Yes
form. blank vs. 88.6 ug a.i./L		0.0143	10.379	<0.001 Yes
form. blank vs. 28.8 ug a.i./L		0.00650	4.718	0.002 Yes
form. blank vs. 7.53 ug a.i./L		0.00170	1.234	1.000 No
form. blank vs. 2.27 ug ai/L		0.000733	0.532	1.000 Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between

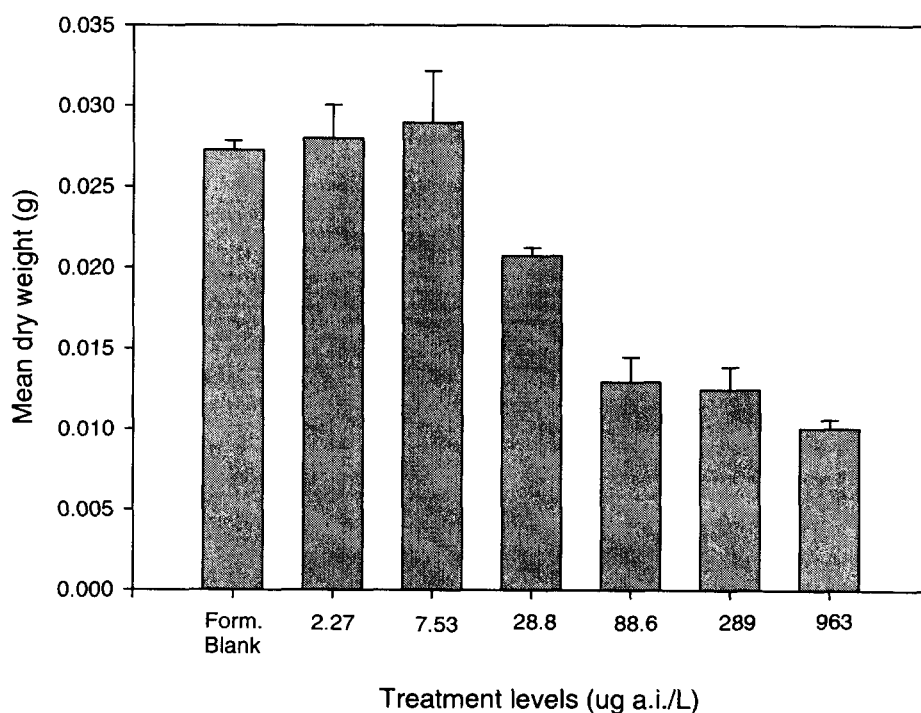
Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants

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means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.



Mean Dry Weight of *Lemna gibba* exposed to Prothioconazole formulation.
Error bars represent the standard deviation. n=3 for all treatments

Conc. ID	1	2	3	4	5	6	7
Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
Response 1	0.0269	0.0301	0.0294	0.0208	0.0127	0.0126	0.0095
Response 2	0.0269	0.0278	0.0255	0.0212	0.0115	0.0110	0.0101

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Response 3 0.02790.02600.03190.02020.01460.01380.0106

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Prothioconazole formulation WEIGHT

Test Start Date: Test Ending Date:

Test Species: *Lemna gibba*

Test Duration: 168 hours

DATA FILE: weight.icp

OUTPUT FILE: weight.i05

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	0.027	0.001	0.028
2	3	2.270	0.028	0.002	0.028
3	3	7.530	0.029	0.003	0.028
4	3	28.800	0.021	0.001	0.021
5	3	88.600	0.013	0.002	0.013
6	3	289.000	0.012	0.001	0.012
7	3	963.000	0.010	0.001	0.010

The Linear Interpolation Estimate: 11.6094 Entered P Value: 5

Number of Resamplings: 80

The Bootstrap Estimates Mean: 11.1725 Standard Deviation: 1.1108

Original Confidence Limits: Lower: 8.6505 Upper: 11.9800

Expanded Confidence Limits: Lower: 5.3956 Upper: 12.3876

Resampling time in Seconds: 0.00 Random_Seed: 977610543

Conc. ID	1	2	3	4	5	6	7
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Conc. Tested	0	2.27	7.53	28.8	88.6	289	963
--------------	---	------	------	------	------	-----	-----

Response 1 0.02690.03010.02940.02080.01270.01260.0095

Response 2 0.02690.02780.02550.02120.01150.0110.0101

Response 3 0.02790.02600.03190.02020.01460.01380.0106

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: Prothioconazole formulation WEIGHT

Test Start Date: Test Ending Date:

Test Species: *Lemna gibba*

Test Duration: 168 hours

DATA FILE: weight.icp

OUTPUT FILE: weight.i50

Conc. ID	Number Replicates	Concentration ug a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	0.027	0.001	0.028
2	3	2.270	0.028	0.002	0.028
3	3	7.530	0.029	0.003	0.028
4	3	28.800	0.021	0.001	0.021
5	3	88.600	0.013	0.002	0.013
6	3	289.000	0.012	0.001	0.012
7	3	963.000	0.010	0.001	0.010

Data Evaluation Report on the acute toxicity of Prothioconazole formulation to aquatic vascular plants

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The Linear Interpolation Estimate: 80.2519 Entered P Value: 50

Number of Resamplings: 80

The Bootstrap Estimates Mean: 81.8988 Standard Deviation: 8.5787

Original Confidence Limits: Lower: 72.7805 Upper: 101.8685

Expanded Confidence Limits: Lower: 64.5620 Upper: 125.6468

Resampling time in Seconds: 0.00 Random_Seed: -1246999841