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

PMRA Submission #:2004-0843   EPA MRID#: 46246101

Data Requirement:	PMRA Data Code:	9.8.5
	EPA DP Barcode:	D303488
	OECD Data Point:	IIA 8.6.1
	EPA MRID:	46246101
	EPA Guideline:	123-2

Test material: JAU 6476 Technical   **Purity:** 98.2%

Common name: Prothioconazole

Chemical name: IUPAC: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4,-triazole-3-thione

CAS name: **2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione**

CAS No.: 178928-70-6

Synonyms: JAU6476

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Staff Scientist, Dynamac Corporation   **Date:** 8/18/04

QC Reviewer: Teri Myers   **Signature:**
Staff Scientist, Dynamac Corporation   **Date:** 8/31/04

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EPA/OPP/EFED/ERB-III

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EPA/OPP/EFED/ERB-IV   8-16-05

Secondary Reviewer: Émilie Larivière   **Date:** 9/1/2005
HC, PMRA, EAD   9/1/05

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

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Kern, M.E., Banman, C.S.,and Lam, C.V. 2003. Toxicity of JAU 6476 Technical 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 Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAY002 (J6883701), and sponsored by Bayer CropScience, RTP, NC. Experimental start date January 17, 2003 and experimental termination date January 24, 2003. The final report issued March 3, 2004.

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Prothioconazole at nominal concentrations of 0 (negative and solvent controls), 0.97, 3.24, 10.8, 36.0, 120, and 400 ppb a.i. under static renewal conditions. The measured concentrations were ≤ 0.5 (<LOQ, negative and solvent controls), 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i.. The percent inhibitions for mean frond numbers were 0, 0, 10, 39, 64, and 71% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for dry weights were 10, 4, 8, 43, 55, and 60% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the solvent control. The percent inhibitions for growth rates were 0, 0, 3, 17, 36, and 44% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for areas under the growth curve were 2, -3, 8, 30, 51, and 60% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control. Frond number was the most sensitive endpoint tested, with an EC_{50} of 73 ppb a.i.; the NOAEC was 3.34 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: 3.34 ppb a.i.
LOAEC: 10.4 ppb a.i.
EC_{05}: 1.6 ppb a.i.   95% C.I.: 0.56-4.6 ppb a.i.
EC_{50}/IC_{50}: 73 ppb a.i.   95% C.I.: 51-110 ppb a.i.
Slope: 0.988 ± 0.102

Growth rates (0-7 day):
NOAEC: 3.34 ppb a.i.
LOAEC: 10.4 ppb a.i.
EC_{05}: 14 ppb a.i.   95% C.I.: 2.5-73 ppb a.i.
EC_{50}/IC_{50}: >404 ppb a.i.   95% C.I.: N/A
Slope: 0.981 ± 0.258

Plant biomass (area under the growth curve):
NOAEC: 3.34 ppb a.i.
LOAEC: 10.4 ppb a.i.
EC_{05}: 1.9 ppb a.i.   95% C.I.: 0.59-6.1 ppb a.i.
EC_{50}/IC_{50}: 150 ppb a.i.   95% C.I.: 100-210 ppb a.i.
Slope: 0.868 ± 0.0988

Dry Weights:
NOAEC: <1.01 ppb a.i.
LOAEC: 1.01 ppb a.i.
EC_{05}: 1.3 ppb a.i.   95% C.I.: 0.20-7.9 ppb a.i.
EC_{50}/IC_{50}: 150 ppb a.i.   95% C.I.: 84-250 ppb a.i.
Slope: 0.799 ± 0.132

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 <u>No</u> Data Confidentiality statements were provided. This test was conducted in accordance with 40 CFR Part 160.

A. MATERIALS:

1. Test Material   Prothioconazole (JAU 6476 Technical)

     Description: Light beige powder

     Lot No./Batch No. :   6233/0031

     Purity: 98.2%

     **Stability of Compound
Under Test Conditions:** The new test concentrations (days 0 and 5) were 89-104% of nominal concentrations and the old test concentrations (days 3 and 7) were not detected and 3-27% 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: <u>Lemna gibba.</u>)*
     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: Two preliminary range-finding studies were conducted to determine the nominal test concentrations for the definitive test. The first preliminary test concentrations were 60.0, 120, 270, 610, 1350, and 3000 ppb a.i.. The test concentrations were compared to a pooled control (dilution water control and solvent control). The percent inhibitions for frond counts and dry weights were 52-78% and 47-69%, respectively, in all treatment groups. The second preliminary test concentrations were 2.56, 6.4, 16, 40, and 100 ppb a.i.. The test concentrations were compared to a pooled control (dilution water control and solvent control). The percent inhibitions for frond counts and dry weights were 13-56% and 7-50%, respectively, in all treatment groups. A NOAEC was not observed in either preliminary test.

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)	7 days 20X AAP Medium; same as test. The batch culture was in log phase growth.	
Test system static/static renewal/ renewal rate for static renewal:	Static Renewal Days 3 and 5	*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).*
Incubation facility	Environmental chamber	
Duration of the test	7 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
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.

Parameter	Details	**Remarks** *Criteria*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP Medium 7.6-7.9 8.7-9.0 disodium EDTA NaHCO₃	*EPA recommend the following culture media:* *Modified hoagland's E+ or 20X-AAP.*
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. *EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
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.	
Number of replicates control: solvent control: treatments:	3 3 3	
Number of plants/replicate	Three plant with 16 fronds per replicate.	There were three plants for each treatment level. *EPA requires 5 plants.*
Number of fronds/plant	16 total fronds per replicate	*EPA requires 3 fronds per plant.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative and solvent controls), 0.97, 3.24, 10.8, 36.0, 120, and 400 ppb a.i. ≤0.5 (<LOQ, negative and solvent controls), 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i.	The mean measured test concentrations are based on day 0 and 5 new solutions. *EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	Acetone, 0.5 mL/L	
Method and interval of analytical verification	HPLC; days 0 and 5 (new solutions), and days 3 and 7 (old solutions).	
Test conditions temperature: photoperiod: light intensity and quality:	24.4-25.6°C continuous light 5.1 klux, cool-white fluorescent light	*EPA temperature: 25°C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
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 (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 solvent controls on day 7 were approximately 17-18X frond numbers on day 0.	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The percent inhibitions for mean frond numbers were 0, 0, 10, 39, 64, and 71% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for dry weights were 10, 4, 8, 43, 55, and 60% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the solvent control. The percent inhibitions for growth rates were 0, 0, 3, 17, 36, and 44% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for areas under the growth curve were 2, -3, 8, 30, 51, and 60% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control.

Table 3: Effect of Prothioconazole 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[a]	Mean Area Under the Growth Curve [a]
		7 days	% inhibition at 7 days[a]			
Negative control (dilution water)	16	279	---	0.0304	0.01701	12360
Solvent control	16	281	--	0.0340	0.01705	12520
1.01 (0.97)	16	280	0	0.0304**	0.01702	12172
3.34 (3.24)	16	280	0	0.0325	0.01703	12872
10.4 (10.8)	16	253	10*	0.0314	0.01644	11388*
35.1 (36.0)	16	172	39*	0.0193**	0.01413*	8656*
106.4 (120)	16	101	64*	0.0153**	0.01095*	6044*
404.0 (400)	16	80	71*	0.0138**	0.00958*	5032*
Reference chemical (if used)	N/A					

[a] The treatment groups were compared to the pooled control for all endpoints.
* Statistically different from the pooled control (Dunnett's one tailed test; p<0.05).
**Statistically different from the solvent control (Dunnett's one tailed test; p<0.05). The difference in the 1.01 ppb a.i. treatment group was not biologically relevant.

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.)	3.34	10.4	10.4	3.34
LOAEC (ppb a.i.)	10.4	35.1	35.1	10.4
EC_{50} (ppb a.i.) (95% C.I.)	74	404	>404	404
EC_{25} (ppb a.i.) (95% C.I.)	15.6	Not reported	70.3	Not reported
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 (the pooled controls were used for frond number, growth rate and biomass comparisons, and the solvent control was used for dry weight 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 8.2 computer program. The NOAEC and LOAEC were determined from analyzed data. Nonparametric analyses were conducted for the biomass and dry weight data. Parametric analyses were conducted for the frond counts 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 or Dunnett's test (dry weight). 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 EC_{05} and EC_{50} values were determined using the Probit method via Nuthatch statistical software.

    Number of fronds:
    NOAEC: 3.34 ppb a.i.
    LOAEC: 10.4 ppb a.i.
    EC_{05}: 1.6 ppb a.i.   95% C.I.: 0.56-4.6 ppb a.i.
    EC_{50}/IC_{50}: 73 ppb a.i.   95% C.I.: 51-110 ppb a.i.
    Slope: 0.988±0.102

    Growth rates (0-7 day):
    NOAEC: 3.34 ppb a.i.
    LOAEC: 10.4 ppb a.i.
    EC_{05}: 14 ppb a.i.   95% C.I.: 2.5-73 ppb a.i.

EC_{50}/IC_{50}: >404 ppb a.i.   95% C.I.: N/A
Slope: 0.981±0.258

Plant biomass (area under the growth curve):
NOAEC: 3.34 ppb a.i.
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EC_{50}/IC_{50}: 150 ppb a.i.   95% C.I.: 100-210 ppb a.i.
Slope: 0.868±0.0988

Dry Weights:
NOAEC: <1.01 ppb a.i.
LOAEC: 1.01 ppb a.i.
EC_{05}: 1.3 ppb a.i.   95% C.I.: 0.20-7.9 ppb a.i.
EC_{50}/IC_{50}: 150 ppb a.i.   95% C.I.: 84-250 ppb a.i.
Slope: 0.799±0.132

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:

With the exception of biomass and dry weight, 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 EC_{50} value (73 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, based on an EC_{50} of 73 ppb a.i..

Number of fronds:
NOAEC: 3.34 ppb a.i.
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Plant biomass (area under the growth curve):
NOAEC: 3.34 ppb a.i.
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Dry Weights:
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EC_{50}/IC_{50}: 150 ppb a.i.   95% C.I.: 84-250 ppb a.i.
Slope: 0.799±0.132

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 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.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond count
File: 6101fc   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	154133.833	25688.972	133.442
Within (Error)	17	3272.667	192.510	
Total	23	157406.500		

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

frond count
File: 6101fc   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	280.000	280.000		
2	1.01	279.667	279.667	0.034	
3	3.34	280.000	280.000	0.000	
4	10.4	253.333	253.333	2.718	*
5	35.1	172.000	172.000	11.008	*
6	106.4	100.667	100.667	18.279	*
7	404	80.333	80.333	20.351	*

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

frond count
File: 6101fc   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	1.01	3	26.048	9.3	0.333
3	3.34	3	26.048	9.3	0.000
4	10.4	3	26.048	9.3	26.667
5	35.1	3	26.048	9.3	108.000
6	106.4	3	26.048	9.3	179.333
7	404	3	26.048	9.3	199.667

frond count
File: 6101fc   Transform: NO TRANSFORMATION

```
        WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
-----------------------------------------------------------------------
GROUP                             ORIGINAL    TRANSFORMED    ISOTONIZED
         IDENTIFICATION      N      MEAN         MEAN          MEAN
------   ---------------    ---   ---------   -----------   ----------
   1       GRPS 1&2 POOLED   6    280.000      280.000       280.000
   2                 1.01    3    279.667      279.667       279.833
   3                 3.34    3    280.000      280.000       279.833
   4                 10.4    3    253.333      253.333       253.333
   5                 35.1    3    172.000      172.000       172.000
   6                106.4    3    100.667      100.667       100.667
   7                 404     3     80.333       80.333        80.333
-----------------------------------------------------------------------



frond count
File: 6101fc          Transform: NO TRANSFORMATION

        WILLIAMS TEST  (Isotonic regression model)     TABLE 2 OF 2
-----------------------------------------------------------------------
                   ISOTONIZED   CALC.     SIG      TABLE     DEGREES OF
    IDENTIFICATION    MEAN     WILLIAMS  P=.05   WILLIAMS     FREEDOM
------------------  ---------- --------- ------- ---------- ------------
    GRPS 1&2 POOLED  280.000
              1.01   279.833    0.017                1.74    k= 1, v=17
              3.34   279.833    0.017                1.82    k= 2, v=17
              10.4   253.333    2.718       *        1.85    k= 3, v=17
              35.1   172.000   11.008       *        1.87    k= 4, v=17
             106.4   100.667   18.279       *        1.87    k= 5, v=17
              404     80.333   20.351       *        1.88    k= 6, v=17
-----------------------------------------------------------------------
s =    13.875
Note: df used for table values are approximate when v > 20.

Estimates of EC%
-----------------------------------------------------------------------
Parameter    Estimate      95% Bounds          Std.Err.  Lower Bound
                         Lower    Upper                   /Estimate
EC5            1.6        0.56      4.6          0.22       0.35
EC10           3.7        1.5       8.9          0.18       0.41
EC25          15.         8.3      28.           0.13       0.55
EC50          73.        51.      1.1E+02        0.075      0.70

        Slope =    0.988  Std.Err. =     0.102


!!!Poor fit: p < 0.001 based on DF=      4.00       17.0
-----------------------------------------------------------------------
6101FC : frond count
-----------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
-----------------------------------------------------------------------
     Dose    #Reps.    Obs.    Pred.     Obs.    Pred.    %Change
                       Mean    Mean     -Pred.   %Control

     0.00    6.00     280.     289.     -9.27    100.      0.00
     1.01    3.00     280.     280.    -0.0857    96.7     3.29
     3.34    3.00     280.     263.     17.4      90.8     9.24
    10.4     3.00     253.     231.     22.2      79.9    20.1
    35.1     3.00     172.     181.     -8.57     62.4    37.6
    106.     3.00     101.     126.    -25.6      43.7    56.3
    404.     3.00      80.3     67.1    13.2       23.2    76.8
```

```
biomass
File: 6101b          Transform: NO TRANSFORMATION

                            ANOVA TABLE
----------------------------------------------------------------------------

SOURCE              DF            SS               MS              F
----------------------------------------------------------------------------
Between             6      206402298.000     34400383.000      120.801

Within (Error)     17        4841088.000       284769.882
----------------------------------------------------------------------------
Total              23      211243386.000
----------------------------------------------------------------------------

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



biomass
File: 6101b          Transform: NO TRANSFORMATION

         BONFERRONI T-TEST    -    TABLE 1 OF 2          Ho:Control<Treatment
----------------------------------------------------------------------------
                            TRANSFORMED    MEAN CALCULATED IN
GROUP      IDENTIFICATION      MEAN         ORIGINAL UNITS    T STAT   SIG
-----  -----------------    -----------   ------------------  ------   ---
   1      GRPS 1&2 POOLED   12440.000         12440.000
   2               1.01     12172.000         12172.000        0.710
   3               3.34     12872.000         12872.000       -1.145
   4               10.4     11388.000         11388.000        2.788    *
   5               35.1      8656.000          8656.000       10.028    *
   6              106.4      6044.000          6044.000       16.950    *
   7               404       5032.000          5032.000       19.632    *
----------------------------------------------------------------------------
Bonferroni T table value =  2.65    (1 Tailed Value, P=0.05,  df=17,6)



biomass
File: 6101b          Transform: NO TRANSFORMATION

         BONFERRONI T-TEST    -    TABLE 2 OF 2          Ho:Control<Treatment
----------------------------------------------------------------------------
                         NUM OF   Minimum Sig Diff   % of     DIFFERENCE
GROUP      IDENTIFICATION REPS    (IN ORIG. UNITS)  CONTROL   FROM CONTROL
-----  -----------------  ------  ----------------  -------   ------------
   1      GRPS 1&2 POOLED   6
   2               1.01     3          1001.836       8.1        268.000
   3               3.34     3          1001.836       8.1       -432.000
   4               10.4     3          1001.836       8.1       1052.000
   5               35.1     3          1001.836       8.1       3784.000
   6              106.4     3          1001.836       8.1       6396.000
   7               404      3          1001.836       8.1       7408.000
----------------------------------------------------------------------------



biomass
File: 6101b          Transform: NO TRANSFORMATION

      WILLIAMS TEST  (Isotonic regression model)     TABLE 1 OF 2
```

```
GROUP                                     ORIGINAL      TRANSFORMED    ISOTONIZED
           IDENTIFICATION      N          MEAN            MEAN           MEAN
-------    --------------    ---      ------------    ------------   ------------
   1       GRPS 1&2 POOLED    6        12440.000       12440.000      12481.000
   2                 1.01     3        12172.000       12172.000      12481.000
   3                 3.34     3        12872.000       12872.000      12481.000
   4                 10.4     3        11388.000       11388.000      11388.000
   5                 35.1     3         8656.000        8656.000       8656.000
   6                106.4     3         6044.000        6044.000       6044.000
   7                 404      3         5032.000        5032.000       5032.000
```

biomass
File: 6101b   Transform: NO TRANSFORMATION

         WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

```
                     ISOTONIZED    CALC.     SIG      TABLE      DEGREES OF
     IDENTIFICATION     MEAN      WILLIAMS   P=.05   WILLIAMS     FREEDOM
    ---------------  -----------  ---------  -----   --------   -----------
    GRPS 1&2 POOLED  12481.000
              1.01   12481.000      0.109               1.74    k= 1, v=17
              3.34   12481.000      0.109               1.82    k= 2, v=17
              10.4   11388.000      2.788      *        1.85    k= 3, v=17
              35.1    8656.000     10.028      *        1.87    k= 4, v=17
             106.4    6044.000     16.950      *        1.87    k= 5, v=17
              404     5032.000     19.632      *        1.88    k= 6, v=17
```

s = 533.638
Note: df used for table values are approximate when v > 20.

Estimates of EC%

```
Parameter   Estimate     95% Bounds        Std.Err.   Lower Bound
                       Lower    Upper                  /Estimate
EC5           1.9       0.59      6.1        0.24        0.31
EC10          5.0       1.9       13.        0.20        0.39
EC25          25.       13.       46.        0.13        0.54
EC50        1.5E+02   1.0E+02   2.1E+02      0.073       0.71
```

       Slope =   0.868 Std.Err. =   0.0988

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

6101B : biomass

Observed vs. Predicted Treatment Group Means

```
     Dose    #Reps.     Obs.     Pred.     Obs.     Pred.    %Change
                        Mean     Mean     -Pred.   %Control
     0.00     6.00   1.24e+04  1.28e+04   -332.     100.      0.00
     1.01     3.00   1.22e+04  1.24e+04   -218.     97.0      2.99
     3.34     3.00   1.29e+04  1.18e+04  1.07e+03   92.4      7.63
     10.4     3.00   1.14e+04  1.08e+04    635.     84.2     15.8
     35.1     3.00   8.66e+03  9.03e+03   -370.     70.7     29.3
     106.     3.00   6.04e+03  7.02e+03   -981.     55.0     45.0
     404.     3.00   5.03e+03  4.51e+03    524.     35.3     64.7
```

```
growth rate
File: 6101g          Transform: NO TRANSFORMATION

                               ANOVA TABLE

---------------------------------------------------------------------

SOURCE          DF            SS              MS              F
---------------------------------------------------------------------
Between          6         19509.406       3251.568        207.662

Within (Error)  17           266.183         15.658
---------------------------------------------------------------------
Total           23         19775.589
---------------------------------------------------------------------


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




growth rate
File: 6101g          Transform: NO TRANSFORMATION

      BONFERRONI T-TEST    -    TABLE 1 OF 2            Ho:Control<Treatment
---------------------------------------------------------------------
                        TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION      MEAN       ORIGINAL UNITS     T STAT   SIG
-----  --------------    -----------  ------------------   ------   ---
  1     GRPS 1&2 POOLED    170.300          170.300
  2              1.01      170.210          170.210         0.032
  3              3.34      170.253          170.253         0.017
  4              104       164.393          164.393         2.111
  5              35.1      141.287          141.287        10.369    *
  6              106.4     109.467          109.467        21.741    *
  7              404        95.757           95.757        26.641    *
---------------------------------------------------------------------
Bonferroni T table value =  2.65     (1 Tailed Value, P=0.05,  df=17,6)




growth rate
File: 6101g          Transform: NO TRANSFORMATION

      BONFERRONI T-TEST    -    TABLE 2 OF 2            Ho:Control<Treatment
---------------------------------------------------------------------
                        NUM OF   Minimum Sig Diff  % of      DIFFERENCE
GROUP    IDENTIFICATION  REPS    (IN ORIG. UNITS)  CONTROL   FROM CONTROL
-----  --------------    ------  ----------------  -------   ------------
  1     GRPS 1&2 POOLED    6
  2              1.01      3           7.429          4.4        0.090
  3              3.34      3           7.429          4.4        0.047
  4              104       3           7.429          4.4        5.907
  5              35.1      3           7.429          4.4       29.013
  6              106.4     3           7.429          4.4       60.833
  7              404       3           7.429          4.4       74.543
---------------------------------------------------------------------




growth rate
File: 6101g          Transform: NO TRANSFORMATION

      WILLIAMS TEST   (Isotonic regression model)    TABLE 1 OF 2
```

```
-----------------------------------------------------------------------
GROUP                              ORIGINAL    TRANSFORMED    ISOTONIZED
         IDENTIFICATION      N       MEAN         MEAN          MEAN
------   --------------     ---   -----------  -----------   -----------
   1      GRPS 1&2 POOLED    6      170.300      170.300       170.300
   2                 1.01    3      170.210      170.210       170.232
   3                 3.34    3      170.253      170.253       170.232
   4                  104    3      164.393      164.393       164.393
   5                 35.1    3      141.287      141.287       141.287
   6                106.4    3      109.467      109.467       109.467
   7                  404    3       95.757       95.757        95.757
-----------------------------------------------------------------------
```

growth rate
File: 6101g   Transform: NO TRANSFORMATION

```
          WILLIAMS TEST  (Isotonic regression model)    TABLE 2 OF 2
---------------------------------------------------------------------
                     ISOTONIZED  CALC.     SIG      TABLE      DEGREES OF
       IDENTIFICATION    MEAN    WILLIAMS  P=.05   WILLIAMS     FREEDOM
       --------------  --------- --------- -----   --------    ---------
       GRPS 1&2 POOLED  170.300
                 1.01   170.232    0.024             1.74      k= 1, v=17
                 3.34   170.232    0.024             1.82      k= 2, v=17
                  104   164.393    2.111     *       1.85      k= 3, v=17
                 35.1   141.287   10.369     *       1.87      k= 4, v=17
                106.4   109.467   21.742     *       1.87      k= 5, v=17
                  404    95.757   26.641     *       1.88      k= 6, v=17
---------------------------------------------------------------------
s =    3.957
```
Note: df used for table values are approximate when v > 20.

Estimates of EC%

```
---------------------------------------------------------------------
Parameter   Estimate    95% Bounds        Std.Err.  Lower Bound
                      Lower     Upper                /Estimate
EC5            14.      2.5       73.       0.35       0.19
EC10           32.      9.1    1.1E+02      0.26       0.29
EC25        1.3E+02     70.    2.5E+02      0.13       0.53
EC50        6.4E+02  3.1E+02   1.3E+03      0.15       0.48

        Slope =   0.981  Std.Err. =    0.258
```

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

```
---------------------------------------------------------------------
6101G : growth rate
---------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
---------------------------------------------------------------------
     Dose    #Reps.    Obs.    Pred.     Obs.     Pred.    %Change
                       Mean    Mean     -Pred.   %Control

     0.00    6.00     170.    170.      0.232     100.      0.00
     1.01    3.00     170.    170.      0.649     99.7      0.298
     3.34    3.00     170.    168.      2.32      98.7      1.25
     35.1    3.00     141.    152.     -10.5      89.2     10.8
     104.    3.00     164.    133.      31.6      78.1     21.9
     106.    3.00     109.    132.     -22.9      77.8     22.2
     404.    3.00     95.8    98.4     -2.62      57.8     42.2
```

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

dry weight
File: 6101dw          Transform: NO TRANSFORMATION


                              ANOVA TABLE
       ----------------------------------------------------------------------

SOURCE              DF              SS              MS              F
       ----------------------------------------------------------------------

Between             6            13.809           2.302          88.538

Within (Error)     14             0.361           0.026
       ----------------------------------------------------------------------

Total              20            14.170
       ----------------------------------------------------------------------


   Critical F value =   2.85  (0.05,6,14)
   Since  F > Critical F  REJECT  Ho:All groups equal




dry weight
File: 6101dw          Transform: NO TRANSFORMATION


       DUNNETTS TEST    -    TABLE 1 OF 2              Ho:Control<Treatment
       ----------------------------------------------------------------------
                         TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION     MEAN        ORIGINAL UNITS    T STAT  SIG
-----  --------------    -----------   ------------------  ------  ---
  1      solvent control   3.400            3.400
  2                1.01    3.043            3.043          2.709   *
  3                3.34    3.253            3.253          1.114
  4                10.4    3.140            3.140          1.975
  5                35.1    1.927            1.927         11.191   *
  6               106.4    1.533            1.533         14.178   *
  7                404     1.377            1.377         15.368   *
       ----------------------------------------------------------------------

Dunnett table value =  2.53     (1 Tailed Value, P=0.05,  df=14,6)




dry weight
File: 6101dw          Transform: NO TRANSFORMATION


       DUNNETTS TEST    -    TABLE 2 OF 2              Ho:Control<Treatment
       ----------------------------------------------------------------------
                         NUM OF   Minimum Sig Diff  % of    DIFFERENCE
GROUP    IDENTIFICATION  REPS    (IN ORIG. UNITS)  CONTROL  FROM CONTROL
-----  --------------    ------   ----------------  ------  ------------
  1      solvent control   3
  2                1.01    3          0.333           9.8       0.357
  3                3.34    3          0.333           9.8       0.147
  4                10.4    3          0.333           9.8       0.260
  5                35.1    3          0.333           9.8       1.473
  6               106.4    3          0.333           9.8       1.867
  7                404     3          0.333           9.8       2.023
       ----------------------------------------------------------------------


dry weight
File: 6101dw          Transform: NO TRANSFORMATION
```

```
            WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
      --------------------------------------------------------------------
      GROUP                             ORIGINAL    TRANSFORMED    ISOTONIZED
                 IDENTIFICATION   N       MEAN         MEAN          MEAN
      ------   ----------------  ---   ----------   -----------   -----------
         1       solvent control  3      3.400         3.400         3.400
         2                 1.01   3      3.043         3.043         3.148
         3                 3.34   3      3.253         3.253         3.148
         4                 10.4   3      3.140         3.140         3.140
         5                 35.1   3      1.927         1.927         1.927
         6                106.4   3      1.533         1.533         1.533
         7                  404   3      1.377         1.377         1.377
      --------------------------------------------------------------------
```

dry weight
File: 6101dw   Transform: NO TRANSFORMATION

```
            WILLIAMS TEST  (Isotonic regression model)    TABLE 2 OF 2
      --------------------------------------------------------------------
                         ISOTONIZED   CALC.      SIG     TABLE      DEGREES OF
          IDENTIFICATION    MEAN     WILLIAMS   P=.05   WILLIAMS     FREEDOM
      ----------------   ----------  --------  ------  ----------  -------------
        solvent control    3.400
                   1.01    3.148      1.920       *       1.76      k= 1, v=14
                   3.34    3.148      1.920       *       1.85      k= 2, v=14
                   10.4    3.140      1.983       *       1.88      k= 3, v=14
                   35.1    1.927     11.238       *       1.89      k= 4, v=14
                  106.4    1.533     14.239       *       1.90      k= 5, v=14
                    404    1.377     15.434       *       1.91      k= 6, v=14
      --------------------------------------------------------------------
```

s =   0.161
Note: df used for table values are approximate when v > 20.

Estimates of EC%
```
      --------------------------------------------------------------------
      Parameter   Estimate     95% Bounds         Std.Err.  Lower Bound
                             Lower     Upper                 /Estimate
      EC5            1.3     0.20       7.9        0.38        0.16
      EC10           3.6     0.82       16.        0.31        0.23
      EC25           21.     8.1        54.        0.20        0.39
      EC50         1.5E+02   84.     2.5E+02       0.11        0.58
```

          Slope =   0.799 Std.Err. =   0.132

!!!Poor fit: p < 0.001 based on DF=   4.00   17.0
```
      --------------------------------------------------------------------
```
6101DW : dry weight
```
      --------------------------------------------------------------------
```
Observed vs. Predicted Treatment Group Means
```
      --------------------------------------------------------------------
         Dose     #Reps.    Obs.     Pred.      Obs.      Pred.    %Change
                           Mean      Mean      -Pred.    %Control

         0.00     6.00     3.22      3.30     -0.0795    100.       0.00
         1.01     3.00     3.04      3.16     -0.117      95.8      4.22
         3.34     3.00     3.25      2.99      0.267      90.5      9.50
         10.4     3.00     3.14      2.71      0.434      82.0     18.0
         35.1     3.00     1.93      2.27     -0.348      68.9     31.1
         106.     3.00     1.53      1.79     -0.260      54.4     45.6
```

404.   3.00   1.38   1.19   0.183   36.2   63.8

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA   Date: September 1, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Aquatic Vascular Plants (*Lemna gibba*)

Kern, M.E., Banman, C.S.,and Lam, C.V. 2003. Toxicity of JAU 6476 Technical 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. EBJAY002 (J6883701), and sponsored by Bayer CropScience, RTP, NC. Report No. 200488. Experimental start date January 17, 2003 and experimental termination date January 24, 2003. The final report issued March 3, 2004.

PMRA DATA CODE: 9.8.5
EPA DP Barcode: D303488
OECD Data Point: IIA 8.6.1
EPA MRID: 46246101
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 prothioconazole (JAU6476, purity 98.2%) at nominal concentrations of 0 (negative and solvent controls), 0.97, 3.24, 10.8, 36.0, 120, and 400 µg a.i./L under static renewal conditions. The measured concentrations were ≤0.5 (<LOQ, negative and solvent controls), 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 µg a.i./L. The study was conducted according to U.S. EPA OPPTS 850.4400, and was in compliance with U.S. EPA 40 CFR Part 160. The percent inhibitions for mean frond numbers were 0, 0, 10, 39, 64, and 71% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 µg a.i./L treatment groups, respectively, compared to the pooled control. The percent inhibitions for dry weights were 10, 4, 8, 43, 55, and 60% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 µg a.i./L treatment groups, respectively, compared to the solvent control. The percent inhibitions for growth rates were 0, 0, 3, 17, 36, and 44% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 µg a.i./L treatment groups, respectively, compared to the pooled control. The percent inhibitions for biomass (areas under the growth curve) were 2, -3, 8, 30, 51, and 60% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 µg a.i./L treatment groups, respectively, compared to the pooled control. The EC_{50} values (95% confidence intervals) determined by the EAD reviewer were 67.1 (58.6-74.0), >404, 101.0 (96.2-105.2), and 76.2 (64.8-88.7) µg a.i./L, for frond numbers, growth rate, biomass and dry weights, respectively; corresponding NOEC values

were 3.34, 10.4, 3.34 and 10.4 µg a.i./L.

Results Synopsis, as determined by EAD reviewer

Test Organism: *Lemna gibba* G3
Test Type: Static Renewal

Number of fronds:
NOEC: 3.34 µg a.i./L
LOEC: 10.4 µg a.i./L
EC_{05}: 7.0 µg a.i./L   95% C.I.: 1.4-8.7 µg a.i./L
EC_{50}/IC_{50} :

Growth rates (0-7 day):
NOEC: 10.4 µg a.i./L
LOEC: 35.1 µg a.i./L
EC_{05}: 13.19 µg a.i./L   95% C.I.: 9.96-15.05 µg a.i./L
EC_{50}/IC_{50} : >404 µg a.i./L   95% C.I.: N/A

Plant biomass (area under the growth curve):
NOEC: 3.34 µg a.i./L
LOEC: 10.4 µg a.i./L
EC_{05}: 7.4 µg a.i./L   95% C.I.: 6.1-9.1 µg a.i./L
EC_{50}/IC_{50} : 101.0 µg a.i./L   95% C.I.: 96.2-105.2 µg a.i./L

Dry Weights:
NOEC: 10.4 µg a.i./L
LOEC: 35.1 µg a.i./L
EC_{05}: 0.7 µg a.i./L   95% C.I.: 0.5-11.5 µg a.i./L
EC_{50}/IC_{50} : 76.2 µg a.i./L   95% C.I.: 64.8-88.7 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 and synonym) 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 (0.017012 for the negative control, 0.017048 for the solvent control and 0.01703 for the pooled controls), the doubling time is 40.7 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.

Data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances), 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 and solvent controls.

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.

4. The EAD reviewer obtained similar results than the study authors and the EPA reviewer for fron numbers and biomass (area under the growth curve). The EAD reviewer agrees with the study authors that the NOEC for dry weight should be 10.4 µg a.i./L, as opposed to the <1.01 µg a.i./L determined by the EPA reviewer. The lack of significant differences at the 3.34 and 10.4 µg a.i./L treatment levels indicate that the statistically significant effect observed at the 1.01 µg a.i./L treatment level was not treatment-related.

5. The EAD reviewer observed a significant inhibition in growth rate at the 35.1 µg a.i./L and higher treatment levels. The 3% inhibition in growth rate observed at the 10.4 µg a.i./L treatment level was not statistically significant, contrary to the results obtained by the EPA reviewer with the use of a Williams test. The EAD reviewer believes the NOEC should be 10.4 µg a.i./L, as also determined by the study author.

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.

Statistical analyses of the EAD reviewer

Frond numbers:

```
Conc. ID         1    2    3    4    5    6    7
----------------------------------------------------
Conc. Tested    00.101 3.34 10.4 35.1106.4  404
----------------------------------------------------
Response  1     292  286  303  259  184  100   89
Response  2     272  294  260  244  169  103   70
Response  3     273  259  277  257  163   99   82
Response  4     261
Response  5     280
Response  6     302
----------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole  FROND
Test Start Date:    Test Ending Date:
Test Species: Lemna
Test Duration:          168 hrs
DATA FILE: aifrond.icp
OUTPUT FILE: aifrond.i05
---------------------------------------------------------------------------
Conc.      Number      Concentration      Response       Std.       Pooled
 ID      Replicates     ug a.i./L          Means        Dev.   Response Means
---------------------------------------------------------------------------
  1          6             0.000           280.000     14.846     280.000
  2          3             0.101           279.667     18.339     279.833
  3          3             3.340           280.000     21.656    ,279.833
  4          3            10.400           253.333      8.145     253.333
  5          3            35.100           172.000     10.817     172.000
  6          3           106.400           100.667      2.082     100.667
  7          3           404.000            80.333      9.609      80.333
---------------------------------------------------------------------------
The Linear Interpolation Estimate:    7.0254    Entered P Value:  5
---------------------------------------------------------------------------
Number of Resamplings:    80
The Bootstrap Estimates Mean:    6.0655 Standard Deviation:      1.9473
Original Confidence Limits:    Lower:      1.3895 Upper:     8.6938
Expanded Confidence Limits:    Lower:     -0.3013 Upper:     9.1944
Resampling time in Seconds:      0.00  Random_Seed: -1345127637
```

```
Conc. ID         1    2    3    4    5    6    7
----------------------------------------------------
Conc. Tested    00.101 3.34 10.4 35.1106.4  404
----------------------------------------------------
Response  1     292  286  303  259  184  100   89
Response  2     272  294  260  244  169  103   70
Response  3     273  259  277  257  163   99   82
Response  4     261
Response  5     280
Response  6     302
----------------------------------------------------
```

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole   FROND
Test Start Date:    Test Ending Date:
Test Species: Lemna
Test Duration:        168 hrs
DATA FILE: aifrond.icp
OUTPUT FILE: aifrond.i50
------------------------------------------------------------------------
Conc.     Number    Concentration    Response    Std.       Pooled
 ID      Replicates    ug a.i./L       Means      Dev.   Response Means
------------------------------------------------------------------------
  1          6           0.000        280.000    14.846     280.000
  2          3           0.101        279.667    18.339     279.833
  3          3           3.340        280.000    21.656     279.833
  4          3          10.400        253.333     8.145     253.333
  5          3          35.100        172.000    10.817     172.000
  6          3         106.400        100.667     2.082     100.667
  7          3         404.000         80.333     9.609      80.333
------------------------------------------------------------------------
The Linear Interpolation Estimate:   67.0850   Entered P Value: 50
------------------------------------------------------------------------
Number of Resamplings:    80
The Bootstrap Estimates Mean:  66.2268 Standard Deviation:     3.8074
Original Confidence Limits:    Lower:    58.5588 Upper:    74.0336
Expanded Confidence Limits:    Lower:    56.0009 Upper:    76.1181
Resampling time in Seconds:     0.00  Random_Seed: -140174165
```

Dry Weight

```
Conc. ID        1     2     3     4     5     6     7
------------------------------------------------------------
Conc. Tested    0 1.01 3.34 10.4 35.1106.4  404
------------------------------------------------------------
Response  1 0.03380.03150.0320.02930.01920.01550.0154
Response  2 0.03280.03150.03360.03420.01950.0160.0112
Response  3 0.03540.02830.0320.03070.01910.01450.0147
------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole
Test Start Date:    Test Ending Date:
Test Species: Lemna gibba
Test Duration:        168 hours
DATA FILE: aiweight.icp
OUTPUT FILE: aiweight.i05
------------------------------------------------------------------------
Conc.     Number    Concentration    Response    Std.       Pooled
 ID      Replicates     ug ai/L        Means      Dev.   Response Means
------------------------------------------------------------------------
  1          3           0.000         0.034     0.001      0.034
  2          3           1.010         0.030     0.002      0.031
  3          3           3.340         0.033     0.001      0.031
  4          3          10.400         0.031     0.003      0.031
  5          3          35.100         0.019     0.000      0.019
  6          3         106.400         0.015     0.001      0.015
```

```
    7            3           404.000          0.014      0.002      0.014
------------------------------------------------------------------------
The Linear Interpolation Estimate:     0.6823   Entered P Value:  5
------------------------------------------------------------------------
Number of Resamplings:    80
The Bootstrap Estimates Mean:   2.7639 Standard Deviation:     3.8885
Original Confidence Limits:    Lower:      0.4898 Upper:    11.4770
Expanded Confidence Limits:    Lower:      0.2781 Upper:    23.3512
Resampling time in Seconds:      0.06  Random_Seed: 2101222003




Conc. ID         1    2    3    4    5    6    7
----------------------------------------------------
Conc. Tested    0 1.01 3.34 10.4 35.1106.4  404
----------------------------------------------------
Response  1 0.03380.03150.0320.02930.01920.01550.0154
Response  2 0.03280.03150.03360.03420.01950.0160.0112
Response  3 0.03540.02830.0320.03070.01910.01450.0147
----------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole
Test Start Date:    Test Ending Date:
Test Species: Lemna gibba
Test Duration:        168 hours
DATA FILE: aiweight.icp
OUTPUT FILE: aiweight.i50
-----------------------------------------------------------------------
Conc.      Number    Concentration    Response     Std.        Pooled
 ID      Replicates      ug ai/L        Means      Dev.    Response Means
-----------------------------------------------------------------------
   1          3           0.000          0.034      0.001       0.034
   2          3           1.010          0.030      0.002       0.031
   3          3           3.340          0.033      0.001       0.031
   4          3          10.400          0.031      0.003       0.031
   5          3          35.100          0.019      0.000       0.019
   6          3         106.400          0.015      0.001       0.015
   7          3         404.000          0.014      0.002       0.014
-----------------------------------------------------------------------
The Linear Interpolation Estimate:     76.1881   Entered P Value: 50
-----------------------------------------------------------------------
Number of Resamplings:    80
The Bootstrap Estimates Mean:   76.4900 Standard Deviation:     6.6615
Original Confidence Limits:    Lower:     64.7558 Upper:    88.7385
Expanded Confidence Limits:    Lower:     52.1801 Upper:   102.5440
Resampling time in Seconds:      0.05  Random_Seed: 107242435
```

Biomass (Area under the growth curve)

```
Conc. ID         1    2    3    4    5    6    7
----------------------------------------------------
Conc. Tested    0 1.01 3.34 10.4 35.1106.4  404
----------------------------------------------------
Response  1 1269612528136201170011700 9168 6072 5784
Response  2 1222812696124561 11136 8412 6072 4308
Response  3 1215611292125401 11328 8388 5988 5004
Response  4 11796
```

```
Response  5 12588
Response  6 13176
-----------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: prothioconazole Biomass
Test Start Date:    Test Ending Date:
Test Species: Lemna gibba
Test Duration:       168 hours
DATA FILE: biomass.icp
OUTPUT FILE: biomass.i05
---------------------------------------------------------------------

Conc.    Number   Concentration    Response     Std.        Pooled
 ID     Replicates    ug a.i./L      Means       Dev.   Response Means
------------------------------------------------------------------------
   1         6            0.000    12440.000   483.626   12481.000
   2         3            1.010    12172.000   766.718   12481.000
   3         3            3.340    12872.000   649.147   12481.000
   4         3           10.400    11388.000   286.747   11388.000
   5         3           35.100     8656.000   443.567    8656.000
   6         3          106.400     6044.000    48.497    6044.000
   7         3          404.000     5032.000   738.398    5032.000
------------------------------------------------------------------------
The Linear Interpolation Estimate:    7.3709   Entered P Value:  5
---------------------------------------------------------------------

Number of Resamplings:   80
The Bootstrap Estimates Mean:   7.2468 Standard Deviation:     0.8603
Original Confidence Limits:   Lower:     6.0569 Upper:     9.1113
Expanded Confidence Limits:   Lower:     5.6627 Upper:     9.6334
Resampling time in Seconds:      0.00   Random_Seed: 1463279087


Conc. ID         1    2    3    4    5    6    7
-----------------------------------------------------
Conc. Tested     0 1.01 3.34 10.4 35.1106.4  404
-----------------------------------------------------
Response  1 12696125281362011700 9168 6072 5784
Response  2 12228126961245611136 8412 6072 4308
Response  3 12156112921254011328 8388 5988 5004
Response  4 11796
Response  5 12588
Response  6 13176
-----------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: prothioconazole Biomass
Test Start Date:    Test Ending Date:
Test Species: Lemna gibba
Test Duration:       168 hours
DATA FILE: biomass.icp
OUTPUT FILE: biomass.i50
-------------------------------------------------------------------

Conc.    Number   Concentration    Response     Std.        Pooled
 ID     Replicates    ug a.i./L      Means       Dev.   Response Means
------------------------------------------------------------------------
   1         6            0.000    12440.000   483.626   12481.000
   2         3            1.010    12172.000   766.718   12481.000
   3         3            3.340    12872.000   649.147   12481.000
   4         3           10.400    11388.000   286.747   11388.000
   5         3           35.100     8656.000   443.567    8656.000
```

```
6          3          106.400      6044.000      48.497    6044.000
7          3          404.000      5032.000     738.398    5032.000
-----------------------------------------------------------------------
The Linear Interpolation Estimate:   101.0361   Entered P Value: 50
-----------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean: 100.3608 Standard Deviation:      2.4151
Original Confidence Limits:   Lower:    96.1582 Upper:   105.2035
Expanded Confidence Limits:   Lower:    94.6948 Upper:   106.4537
Resampling time in Seconds:    0.00  Random_Seed: -302385297
```

Growth Rate

```
Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------------------
Conc. Tested    0 1.01 3.34 10.4 35.1106.4   404
-----------------------------------------------------------
Response  1 0.0172870.0171630.0175070.0165730.0145380.0109080.010215
Response  2 0.0168640.0173270.0165960.0162180.0140320.0110840.008785
Response  3 0.0168860.0165730.0169730.0165270.0138160.0108480.009727
Response  4 0.016619
Response  5 0.017037
Response  6 0.017487
-----------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole 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.    Number    Concentration    Response    Std.       Pooled
 ID     Replicates    ug a.i./L       Means      Dev.    Response Means
-----------------------------------------------------------------------
  1         6          0.000          0.017      0.000      0.017
  2         3          1.010          0.017      0.000      0.017
  3         3          3.340          0.017      0.000      0.017
  4         3         10.400          0.016      0.000      0.016
  5         3         35.100          0.014      0.000      0.014
  6         3        106.400          0.011      0.000      0.011
  7         3        404.000          0.010      0.001      0.010
-----------------------------------------------------------------------
The Linear Interpolation Estimate:    13.1882   Entered P Value:  5
-----------------------------------------------------------------------
Number of Resamplings:    80
The Bootstrap Estimates Mean:   12.9370 Standard Deviation:     1.4206
Original Confidence Limits:    Lower:     9.9608 Upper:    15.0497
Expanded Confidence Limits:    Lower:     8.9926 Upper:    15.6082
Resampling time in Seconds:     0.05  Random_Seed: -558238660


Conc. ID        1    2    3    4    5    6    7
-----------------------------------------------------------
Conc. Tested    0 1.01 3.34 10.4 35.1106.4   404
-----------------------------------------------------------
Response  1 0.0172870.0171630.0175070.0165730.0145380.0109080.010215
```

```
Response  2 0.0168640.0173270.0165960.0162180.0140320.0110840.008785
Response  3 0.0168860.0165730.0169730.0165270.0138160.0108480.009727
Response  4 0.016619
Response  5 0.017037
Response  6 0.017487
-------------------------------------------------
```

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole 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	0.017	0.000	0.017
2	3	1.010	0.017	0.000	0.017
3	3	3.340	0.017	0.000	0.017
4	3	10.400	0.016	0.000	0.016
5	3	35.100	0.014	0.000	0.014
6	3	106.400	0.011	0.000	0.011
7	3	404.000	0.010	0.001	0.010

*** 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.