Data Evaluation Report on the acute toxicity of Prothioconazole metabolite, JAU6476-desthio, to aquatic vascular plants *Lemna gibba*

PMRA Submission #:2004-0843 EPA MRID#: 46246104

Data Requirement:

PMRA Data Code:	9.8.5
EPA DP Barcode:	D303488
OECD Data Point:	IIA 8.6.1
EPA MRID:	46246104
EPA Guideline:	123-2

Test material: JAU 6476-Desthio **Purity:** 97.0%
Common name: Prothioconazole metabolite
Chemical name: IUPAC: alpha-(1-Chlorocyclopropyl)-alpha-[(2-chlorophenyl)methyl]-1H-1,2,4-triazole-ethanol
CAS name: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS No.: 120983-64-4
Synonyms: JAU 6476 metabolite

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8/30/05

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-Desthio 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 Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX084 (J6883702), and sponsored by Bayer CropScience, RTP, NC. Experimental start date December 6 and experimental termination date December 13, 2003. The final report issued December 18, 2003.

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Prothioconazole metabolite at nominal concentrations of 0 (negative and solvent controls), 2.56, 6.4, 16.0, 40.0, and 100 ppb under static renewal conditions. The measured concentrations were ≤0.5 (<LOQ, negative and solvent controls), 2.42, 5.78, 14.3, 35.6, and 89.77 ppb. The percent inhibitions for mean frond numbers were -4, 0, 14, 52, and 73% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The percent inhibitions for dry weights were 4, 7, 26, 57, and 61% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the pooled control. The percent inhibitions for growth rates were -2, 0, 6, 30, and 53% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The percent inhibitions for areas under the growth curve were -9, -3, 9, 41, and 62% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The NOAEC for all endpoints was 5.8 ppb. Frond number was the most sensitive endpoint tested, with an EC_{50} of 35 ppb.

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:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 3.9 ppb 95% C.I.: 2.4-6.4 ppb
EC_{50}/IC_{50}: 35 ppb 95% C.I.: 30-41 ppb
Slope: 1.73±0.145

Growth rates (0-7 day):
NOAEC:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 7.5 ppb 95% C.I.: 5.5-10 ppb
EC_{50}/IC_{50}: 76 ppb 95% C.I.: 70-83 ppb
Slope: 1.64±0.104

Plant biomass (area under the growth curve):
NOAEC:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 4.3 ppb 95% C.I.: 2.3-8.2 ppb
EC_{50}/IC_{50}: 47 ppb 95% C.I.: 39-57 ppb
Slope: 1.58±0.172

Dry Weights:
NOAEC:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 1.7 ppb 95% C.I.: 0.6-5.1 ppb
EC_{50}/IC_{50}: 40 ppb 95% C.I.: 29-56 ppb

Slope: 1.20±0.168

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 metabolite (JAU 6476-Desthio)

Description: White powder

Lot No./Batch No. : RUX76-10/8a

Purity: 97.0%

Stability of Compound Under Test Conditions: The new test concentrations (days 0 and 3) were 90-100% of nominal concentrations and the old test concentrations (day 7) were 83-96% of nominal concentrations.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)
Water solubility: 25 mg metabolite/L with solvent load of 0.5 mL of acetone per liter of water.

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

2. Test organism:

Name: Duckweed, *Lemna gibba* *(EPA requires a vascular species: Lemna gibba.)*
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 preliminary range-finding study was conducted to determine the nominal test concentrations for the definitive test. The test concentrations were 1.0, 10, 100, and 1000 ppb. The test concentrations were compared to a pooled control (dilution water control and solvent control). The percent inhibitions for frond counts were 1, 28, 82, and 86% in the 1.0, 10, 100, and 1000 ppb treatment groups, respectively. The dry weight percent inhibitions were -7, 22, 59, and 64% in the 1.0, 10, 100, and 1000 ppb treatment groups, respectively.

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 Day 3	*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.*

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 $NaHCO_3$	*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.	

Parameter	Details	Remarks *Criteria*
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.*
Test concentrations nominal: measured:	0 (negative and solvent controls), 2.56, 6.4, 16.0, 40.0, and 100 ppb ≤0.5 (<LOQ, negative and solvent controls), 2.42, 5.78, 14.3, 35.6, 89.77 ppb	*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 3 (new solutions), and day 7 (old solutions).	
Test conditions temperature: photoperiod: light intensity and quality:	 23.6-25.6°C continuous light 5.2 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 12-14X 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 -4, 0, 14, 52, and 73% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The percent inhibitions for dry weights were 4, 7, 26, 57, and 61% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the pooled control. The percent inhibitions for growth rates were -2, 0, 6, 30, and 53% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The percent inhibitions for areas under the growth curve were -9, -3, 9, 41, and 62% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control.

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

Treatment measured (nominal) concentrations ppb	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	216	---	0.0246	0.01550	10,104
Solvent control	16	187	--	0.0215	0.01462	8,460
2.42 (2.56)	16	195	-4	0.0211	0.01487	9,192
5.78 (6.4)	16	187	0	0.0214	0.01463	8,696
14.3 (16.0)	16	160*	14	0.0170**	0.01369*	7,740*
35.6 (40.0)	16	90*	52	0.0100**	0.01030*	5,020*
89.77 (100)	16	51*	73	0.0090**	0.00693*	3,192*
Reference chemical (if used)	N/A					

[a] The treatment groups were compared to the solvent control for frond number, biomass, and growth rates. The dry weight were compared tot he pooled control.
* Statistically different from the solvent control (Dunnett's one tailed test; $p<0.05$).
** Statistically different from the solvent 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 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)	5.8	5.8	5.8	5.8
LOAEC (ppb)	14.3	14.3	14.3	14.3
EC_{50} (ppb) (95% C.I.)	39.4	41.1	80.9	56.8
EC_{25} (ppb) (95% C.I.)	17.1	10.9	32.4	23.2
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 46. 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. The Logistic Model or Bruce/Versteeg Cumulative Normal Model using nonlinear (weighted) regression analysis was used to estimate the EC50 and EC25 values. 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 a significant difference between the two control groups, the treatment groups were compared to the solvent control. For dry weight, no significant difference was detected between the control groups, so the treatment groups were compared to the pooled control group. 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:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 3.9 ppb 95% C.I.: 2.4-6.4 ppb
EC_{50}/IC_{50}: 35 ppb 95% C.I.: 30-41 ppb
Slope: 1.73±0.145

Growth rates (0-7 day):
NOAEC:5.8 ppb
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Plant biomass (area under the growth curve):
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EC_{05}: 4.3 ppb 95% C.I.: 2.3-8.2 ppb
EC_{50}/IC_{50}: 47 ppb 95% C.I.: 39-57 ppb
Slope: 1.58±0.172

Dry Weights:
NOAEC:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 1.7 ppb 95% C.I.: 0.6-5.1 ppb
EC_{50}/IC_{50}: 40 ppb 95% C.I.: 29-56 ppb
Slope: 1.20±0.168

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 EC_{50} value (35 ppb). 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 EC_{50} of 35 ppb.

Number of fronds:
NOAEC:5.8 ppb
LOAEC: 14.3 ppb
EC_{05}: 3.9 ppb 95% C.I.: 2.4-6.4 ppb
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EC_{50}/IC_{50}: 40 ppb 95% C.I.: 29-56 ppb
Slope: 1.20±0.168

Most Sensitive Endpoint: Frond number

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond count
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	53931.333	10786.267	175.704
Within (Error)	12	736.667	61.389	
Total	17	54668.000		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

frond count
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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	186.667	186.667		
2	2.4	195.000	195.000	-1.303	
3	5.8	187.000	187.000	-0.052	
4	14.3	159.667	159.667	4.221	*
5	35.6	90.333	90.333	15.058	*
6	89.8	51.333	51.333	21.155	*

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=12,5)

frond count
File: 6104fc 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	solvent control	3			
2	2.4	3	17.151	9.2	-8.333
3	5.8	3	17.151	9.2	-0.333
4	14.3	3	17.151	9.2	27.000
5	35.6	3	17.151	9.2	96.333
6	89.8	3	17.151	9.2	135.333

frond count
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	3	186.667	186.667	190.833
2	2.4	3	195.000	195.000	190.833
3	5.8	3	187.000	187.000	187.000
4	14.3	3	159.667	159.667	159.667
5	35.6	3	90.333	90.333	90.333
6	89.8	3	51.333	51.333	51.333

frond count
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	190.833				
2.4	190.833	0.651		1.78	k= 1, v=12
5.8	187.000	0.052		1.87	k= 2, v=12
14.3	159.667	4.221	*	1.90	k= 3, v=12
35.6	90.333	15.058	*	1.92	k= 4, v=12
89.8	51.333	21.155	*	1.93	k= 5, v=12

s = 7.835
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	3.9	2.4	6.4	0.10	0.61
EC10	6.3	4.2	9.6	0.086	0.66
EC25	14.	11.	19.	0.059	0.75
EC50	35.	30.	41.	0.035	0.85

Slope = 1.73 Std.Err. = 0.145

Goodness of fit: p = 0.16 based on DF= 3.0 15.

6104FC : frond count

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	202.	202.	-0.761	100.	0.00
2.40	3.00	195.	198.	-2.79	97.8	2.21
5.80	3.00	187.	184.	2.67	91.1	8.87
14.3	3.00	160.	151.	8.21	74.9	25.1
35.6	3.00	90.3	99.9	-9.61	49.4	50.6
89.8	3.00	51.3	48.3	3.03	23.9	76.1

biomass
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	86300232.000	17260046.400	96.034
Within (Error)	12	2156736.000	179728.000	
Total	17	88456968.000		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

biomass
File: 6104b Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	8460.000	8460.000		
2	2.4	9192.000	9192.000	-2.115	
3	5.8	8696.000	8696.000	-0.682	
4	14.3	7740.000	7740.000	2.080	
5	35.6	5020.000	5020.000	9.938	*
6	89.8	3192.000	3192.000	15.219	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

biomass
File: 6104b Transform: NO TRANSFORMATION

DUNNETTS 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	solvent control	3			
2	2.4	3	865.371	10.2	-732.000
3	5.8	3	865.371	10.2	-236.000
4	14.3	3	865.371	10.2	720.000
5	35.6	3	865.371	10.2	3440.000
6	89.8	3	865.371	10.2	5268.000

biomass
File: 6104b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	solvent control	3	8460.000	8460.000	8826.000
2	2.4	3	9192.000	9192.000	8826.000
3	5.8	3	8696.000	8696.000	8696.000
4	14.3	3	7740.000	7740.000	7740.000
5	35.6	3	5020.000	5020.000	5020.000
6	89.8	3	3192.000	3192.000	3192.000

biomass
File: 6104b 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
solvent control	8826.000				
2.4	8826.000	1.057		1.78	k= 1, v=12
5.8	8696.000	0.682		1.87	k= 2, v=12
14.3	7740.000	2.080	*	1.90	k= 3, v=12
35.6	5020.000	9.938	*	1.92	k= 4, v=12
89.8	3192.000	15.219	*	1.93	k= 5, v=12

s = 423.943
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	4.3	2.3	8.2	0.13	0.53
EC10	7.3	4.3	12.	0.11	0.59
EC25	18.	13.	25.	0.071	0.71
EC50	47.	39.	57.	0.040	0.82

Slope = 1.58 Std.Err. = 0.172

Goodness of fit: p = 0.49 based on DF= 3.0 15.

6104B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	9.28e+03	9.35e+03	-69.2	100.	0.00
2.40	3.00	9.19e+03	9.16e+03	30.6	98.0	2.03
5.80	3.00	8.70e+03	8.65e+03	44.4	92.5	7.48
14.3	3.00	7.74e+03	7.42e+03	316.	79.4	20.6
35.6	3.00	5.02e+03	5.39e+03	-371.	57.6	42.4
89.8	3.00	3.19e+03	3.07e+03	118.	32.9	67.1

growth rate
File: 6104g Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	15595.606	3119.121	306.186
Within (Error)	12	122.244	10.187	
Total	17	15717.850		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

growth rate
File: 6104g Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	146.180	146.180		
2	2.4	148.747	148.747	-0.985	
3	5.8	146.340	146.340	-0.061	
4	14.3	136.877	136.877	3.570	*
5	35.6	103.013	103.013	16.564	*
6	89.8	69.267	69.267	29.514	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

growth rate
File: 6104g Transform: NO TRANSFORM

DUNNETTS 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	solvent control	3			
2	2.4	3	6.515	4.5	-2.567
3	5.8	3	6.515	4.5	-0.160
4	14.3	3	6.515	4.5	9.303
5	35.6	3	6.515	4.5	43.167
6	89.8	3	6.515	4.5	76.913

growth rate
File: 6104g Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	3	146.180	146.180	147.463
2	2.4	3	148.747	148.747	147.463

3	5.8	3	146.340	146.340	146.340
4	14.3	3	136.877	136.877	136.877
5	35.6	3	103.013	103.013	103.013
6	89.8	3	69.267	69.267	69.267

growth rate
File: 6104g Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	147.463				
2.4	147.463	0.492		1.78	k= 1, v=12
5.8	146.340	0.061		1.87	k= 2, v=12
14.3	136.877	3.570	*	1.90	k= 3, v=12
35.6	103.013	16.564	*	1.92	k= 4, v=12
89.8	69.267	29.514	*	1.93	k= 5, v=12

s = 3.192
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	7.5	5.5	10.	0.065	0.73
EC10	13.	9.8	16.	0.051	0.78
EC25	29.	25.	34.	0.030	0.86
EC50	76.	70.	83.	0.018	0.92

Slope = 1.64 Std.Err. = 0.104

Goodness of fit: p = 0.21 based on DF= 3.0 15.

6104G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	151.	151.	-0.320	100.	0.00
2.40	3.00	149.	150.	-1.09	99.3	0.697
5.80	3.00	146.	146.	0.514	96.6	3.36
14.3	3.00	137.	133.	3.68	88.3	11.7
35.6	3.00	103.	106.	-3.41	70.5	29.5
89.8	3.00	69.3	68.3	0.946	45.3	54.7

dry weight
File: 6104dw Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F

Between	5	6.786	1.357	52.192
Within (Error)	15	0.383	0.026	
Total	20	7.169		

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

dry weight
File: 6104dw Transform: NO TRANSFORM

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	2.307	2.307		
2	2.4	2.213	2.213	0.819	
3	5.8	2.143	2.143	1.433	
4	14.3	1.697	1.697	5.350	*
5	35.6	0.997	0.997	11.489	*
6	89.8	0.903	0.903	12.308	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

dry weight
File: 6104dw Transform: NO TRANSFORM

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	2.4	3	0.297	12.9	0.093
3	5.8	3	0.297	12.9	0.163
4	14.3	3	0.297	12.9	0.610
5	35.6	3	0.297	12.9	1.310
6	89.8	3	0.297	12.9	1.403

dry weight
File: 6104dw Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	2.307	2.307	2.307
2	2.4	3	2.213	2.213	2.213
3	5.8	3	2.143	2.143	2.143
4	14.3	3	1.697	1.697	1.697
5	35.6	3	0.997	0.997	0.997

6	89.8	3	0.903	0.903	0.903

dry weight
File: 6104dw Transform: NO TRANSFORM

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	2.307				
2.4	2.213	0.826		1.75	k= 1, v=15
5.8	2.143	1.445		1.84	k= 2, v=15
14.3	1.697	5.397	*	1.87	k= 3, v=15
35.6	0.997	11.590	*	1.88	k= 4, v=15
89.8	0.903	12.416	*	1.89	k= 5, v=15

s = 0.160
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.7	0.60	5.1	0.22	0.34
EC10	3.5	1.5	8.4	0.18	0.42
EC25	11.	6.3	20.	0.12	0.56
EC50	40.	29.	56.	0.068	0.72

Slope = 1.20 Std.Err. = 0.168

!!!Poor fit: p = 0.0057 based on DF= 3.0 15.

6104DW : dry weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	2.31	2.35	-0.0433	100.	0.00
2.40	3.00	2.21	2.19	0.0273	93.0	6.98
5.80	3.00	2.14	1.99	0.157	84.5	15.5
14.3	3.00	1.70	1.66	0.0356	70.7	29.3
35.6	3.00	0.997	1.24	-0.241	52.7	47.3
89.8	3.00	0.903	0.795	0.108	33.8	66.2

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

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 30, 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-Desthio 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. EBJAX084 (J6883702), and sponsored by Bayer CropScience, RTP, NC. Experimental start date December 6 and experimental termination date December 13, 2003. The final report issued December 18, 2003.

PMRA DATA CODE: 9.8.5
EPA DP Barcode: D303488
OECD Data Point: IIA 8.6.1
EPA MRID: 46246104
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 transformation product JAU6476-desthio (purity: 97%) at nominal concentrations of 0 (negative and solvent controls), 2.56, 6.4, 16.0, 40.0, and 100 µg JAU6476-desthio/L under static renewal conditions. The measured concentrations were ≤0.5 (<LOQ, negative and solvent controls), 2.42, 5.78, 14.3, 35.6, and 89.77 µg JAU6476-desthio/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 -4, 0, 14, 52, and 73% in the 2.42, 5.78, 14.30, 65.60, and 89.77 µg JAU6476-desthio/L treatment groups, respectively, compared to the solvent control. The percent inhibitions for dry weights were 4, 7, 26, 57, and 61% in the 2.42, 5.78, 14.30, 65.60, and 89.77 µg JAU6476-desthio/L treatment groups, respectively, compared to the pooled control. The percent inhibitions for growth rates were -2, 0, 6, 30, and 53% in the 2.42, 5.78, 14.30, 65.60, and 89.77 µg JAU6476-desthio/L treatment groups, respectively, compared to the solvent control. The percent inhibitions for biomass (area under the growth curve) were -9, -3, 9, 41, and 62% in the 2.42, 5.78, 14.30, 65.60, and 89.77 µg JAU6476-desthio/L treatment groups, respectively, compared to the solvent control. The NOEC for all endpoints was 5.8 µg JAU6476-desthio/L. The EC_{50} (95% confidence intervals) values, calculated by the EAD reviewer, were 34, 30.8, 82.6 and 53.6 µg JAU6476-desthio/L for mean 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: 5.8 µg JAU6476-desthio/L
LOEC: 14.3 µg JAU6476-desthio/L
EC_{05}: 7.6 µg JAU6476-desthio/L 95% C.I.: 5.3-9.4 µg JAU6476-desthio/L
EC_{50}/IC_{50}: 34 µg JAU6476-desthio/L 95% C.I.: 32.9-35.1 µg JAU6476-desthio/L

Growth rates (0-7 day):
NOEC:5.8 µg JAU6476-desthio/L
LOEC: 14.3 µg JAU6476-desthio/L
EC_{05}: 11.4 µg JAU6476-desthio/L 95% C.I.: 9.2-14.3 µg JAU6476-desthio/L
EC_{50}/IC_{50}: 82.6 µg JAU6476-desthio/L 95% C.I.: 77.2-87.8 µg JAU6476-desthio/L

Plant biomass (area under the growth curve):
NOEC:5.8 µg JAU6476-desthio/L
LOEC: 14.3 µg JAU6476-desthio/L
EC_{05}: 8.6 µg JAU6476-desthio/L 95% C.I.: 5.7-13.5 µg JAU6476-desthio/L
EC_{50}/IC_{50}: 53.6 µg JAU6476-desthio/L 95% C.I.: 47.3-58.6 µg JAU6476-desthio/L

Dry Weights:
NOEC: 5.8 µg JAU6476-desthio/L
LOEC: 14.3 µg JAU6476-desthio/L
EC_{05}: 3.5 µg JAU6476-desthio/L 95% C.I.: 1.3-7.6 µg JAU6476-desthio/L
EC_{50}/IC_{50}: 30.8 µg JAU6476-desthio/L 95% C.I.: 27.8-33.3 µg JAU6476-desthio/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 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 negative and solvent controls (0.015496 for the negative control, 0.014618 for the solvent control), the doubling time is 47.4 and 44.7 hours, respectively, 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.

Based on visual inspection of the data and the results of statistical analyses of the study author and the EPA reviewer, the EAD reviewer did not feel it was necessary to redo the statistical analyses to verify the NOEC for mean frond numbers, dry weight, biomass and growth rate. The EAD reviewer agrees with the conclusions that the NOEC is 5.8 µg JAU6476-desthio/L for all endpoints.

The EC_{vv} 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.

Statistical analyses of the EAD reviewer

Frond Number

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	2.42	5.78	14.3	35.6	89.77
Response 1	182	185	186	166	90	55
Response 2	198	190	187	150	88	52
Response 3	180	210	188	163	93	47

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio FROND NUMBERS
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/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	186.667	9.866	190.833
2	3	2.420	195.000	13.229	190.833
3	3	5.780	187.000	1.000	187.000
4	3	14.300	159.667	8.505	159.667
5	3	35.600	90.333	2.517	90.333
6	3	89.770	51.333	4.041	51.333

```
The Linear Interpolation Estimate:     7.5593   Entered P Value:  5
Number of Resamplings:   80
The Bootstrap Estimates Mean:   7.4975 Standard Deviation:     1.2372
Original Confidence Limits:   Lower:     5.2712 Upper:     9.4471
Expanded Confidence Limits:   Lower:     2.7543 Upper:    11.5237
Resampling time in Seconds:     0.00  Random_Seed: 1838669238
```

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	2.42	5.78	14.3	35.6	89.77
Response 1	182	185	186	166	90	55
Response 2	198	190	187	150	88	52
Response 3	180	210	188	163	93	47

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio FROND NUMBERS
Test Start Date:    Test Ending Date:
Test Species: Lemna gibba
```

```
Test Duration:       168 hours
DATA FILE: frond.icp
OUTPUT FILE: frond.i50
```

Conc. ID	Number Replicates	Concentration ug/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	186.667	9.866	190.833
2	3	2.420	195.000	13.229	190.833
3	3	5.780	187.000	1.000	187.000
4	3	14.300	159.667	8.505	159.667
5	3	35.600	90.333	2.517	90.333
6	3	89.770	51.333	4.041	51.333

```
The Linear Interpolation Estimate:    34.0383   Entered P Value: 50
Number of Resamplings:   80
The Bootstrap Estimates Mean:  33.9423 Standard Deviation:     0.6555
Original Confidence Limits:   Lower:    32.8756 Upper:    35.0830
Expanded Confidence Limits:   Lower:    31.5965 Upper:    36.2321
Resampling time in Seconds:     0.00  Random_Seed: -520608170
```

Dry weight

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	2.42	5.78	14.3	35.6	89.77
Response	1	0.0255	0.0212	0.0208	0.0186	0.0097	0.0097
Response	2	0.0260	0.0226	0.0231	0.0166	0.0107	0.0085
Response	3	0.0224	0.0226	0.0204	0.0157	0.0095	0.0089
Response	4	0.0204					
Response	5	0.0232					
Response	6	0.0209					

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio DRY 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/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.023	0.002	0.023
2	3	2.420	0.022	0.001	0.022
3	3	5.780	0.021	0.001	0.021
4	3	14.300	0.017	0.001	0.017
5	3	35.600	0.010	0.001	0.010
6	3	89.770	0.009	0.001	0.009

```
The Linear Interpolation Estimate:     3.4760   Entered P Value:  5
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   3.9646 Standard Deviation:     2.1763
Original Confidence Limits:   Lower:     1.2833 Upper:     7.5512
Expanded Confidence Limits:   Lower:     0.6255 Upper:     8.7737
Resampling time in Seconds:     0.00  Random_Seed: -1762858746


Conc. ID             1         2         3         4         5         6
---------------------------------------------------------------------
Conc. Tested         0      2.42      5.78      14.3      35.6     89.77
---------------------------------------------------------------------
Response  1     0.0255    0.0212    0.0208    0.0186    0.0097    0.0097
Response  2     0.0260    0.0226    0.0231    0.0166    0.0107    0.0085
Response  3     0.0224    0.0226    0.0204    0.0157    0.0095    0.0089
Response  4     0.0204
Response  5     0.0232
Response  6     0.0209
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio DRY WEIGHT
Test Start Date:    Test Ending Date:
Test Species: Lemna gibba
Test Duration:       168 hours
DATA FILE: weight.icp
OUTPUT FILE: weight.i50
---------------------------------------------------------------------
Conc.     Number     Concentration     Response      Std.       Pooled
 ID     Replicates          ug/L        Means        Dev.   Response Means
---------------------------------------------------------------------
  1          6             0.000         0.023      0.002       0.023
  2          3             2.420         0.022      0.001       0.022
  3          3             5.780         0.021      0.001       0.021
  4          3            14.300         0.017      0.001       0.017
  5          3            35.600         0.010      0.001       0.010
  6          3            89.770         0.009      0.001       0.009
---------------------------------------------------------------------
The Linear Interpolation Estimate:    30.8329   Entered P Value: 50
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:  31.0820 Standard Deviation:     1.5114
Original Confidence Limits:   Lower:    27.8194 Upper:    33.3179
Expanded Confidence Limits:   Lower:    26.9154 Upper:    34.0634
Resampling time in Seconds:     0.00  Random_Seed: -272915178
```

Growth Rate

```
Conc. ID             1         2         3         4         5         6
---------------------------------------------------------------------
Conc. Tested         0      2.42      5.78      14.3      35.6     89.77
---------------------------------------------------------------------
Response  1   0.014473  0.014570  0.014602  0.013925  0.010281  0.007350
```

```
Response  2   0.014974  0.014729  0.014634  0.013322  0.010147  0.007016
Response  3   0.014407  0.015325  0.014666  0.013816  0.010476  0.006414
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio 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/L          Means        Dev.   Response Means
---------------------------------------------------------------------
  1          3              0.000          0.015       0.000       0.015
  2          3              2.420          0.015       0.000       0.015
  3          3              5.780          0.015       0.000       0.015
  4          3             14.300          0.014       0.000       0.014
  5          3             35.600          0.010       0.000       0.010
  6          3             89.770          0.007       0.000       0.007
---------------------------------------------------------------------
The Linear Interpolation Estimate:    11.4068   Entered P Value:  5
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:  11.2552 Standard Deviation:     1.3219
Original Confidence Limits:   Lower:     9.1722 Upper:    14.2903
Expanded Confidence Limits:   Lower:     6.7141 Upper:    17.4622
Resampling time in Seconds:     0.00  Random_Seed: 746059542


Conc. ID             1         2         3         4         5         6
---------------------------------------------------------------------
Conc. Tested         0      2.42      5.78      14.3      35.6     89.77
---------------------------------------------------------------------
Response  1   0.014473  0.014570  0.014602  0.013925  0.010281  0.007350
Response  2   0.014974  0.014729  0.014634  0.013322  0.010147  0.007016
Response  3   0.014407  0.015325  0.014666  0.013816  0.010476  0.006414
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio 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.     Number      Concentration      Response       Std.       Pooled
 ID     Replicates          ug/L          Means        Dev.   Response Means
---------------------------------------------------------------------
  1          3              0.000          0.015       0.000       0.015
  2          3              2.420          0.015       0.000       0.015
  3          3              5.780          0.015       0.000       0.015
  4          3             14.300          0.014       0.000       0.014
  5          3             35.600          0.010       0.000       0.010
  6          3             89.770          0.007       0.000       0.007
---------------------------------------------------------------------
```

```
The Linear Interpolation Estimate:    82.6028   Entered P Value: 50
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:  82.0151 Standard Deviation:     2.9201
Original Confidence Limits:   Lower:    77.1590 Upper:    87.7935
Expanded Confidence Limits:   Lower:    71.1709 Upper:    93.5032
Resampling time in Seconds:     0.06  Random_Seed: 749285302
```

Biomass (Area under the growth curve)

```
Conc. ID             1         2         3         4         5         6
---------------------------------------------------------------------
Conc. Tested         0      2.42      5.78      14.3      35.6     89.77
---------------------------------------------------------------------
Response  1       8364      8796      8580      8340      5052      3408
Response  2       8652      8652      8760      7224      5148      3156
Response  3       8364     10128      8748      7656      4860      3012
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio 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/L        Means        Dev.    Response Means
---------------------------------------------------------------------
  1          3             0.000      8460.000    166.277    8826.000
  2          3             2.420      9192.000    813.791    8826.000
  3          3             5.780      8696.000    100.638    8696.000
  4          3            14.300      7740.000    562.722    7740.000
  5          3            35.600      5020.000    146.642    5020.000
  6          3            89.770      3192.000    200.440    3192.000
---------------------------------------------------------------------
The Linear Interpolation Estimate:     8.5543   Entered P Value:  5
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   8.6822 Standard Deviation:     1.9922
Original Confidence Limits:   Lower:     5.6832 Upper:    13.5274
Expanded Confidence Limits:   Lower:     2.5249 Upper:    18.9978
Resampling time in Seconds:     0.00  Random_Seed: -2088775434

Conc. ID             1         2         3         4         5         6
---------------------------------------------------------------------
Conc. Tested         0      2.42      5.78      14.3      35.6     89.77
---------------------------------------------------------------------
Response  1       8364      8796      8580      8340      5052      3408
Response  2       8652      8652      8760      7224      5148      3156
Response  3       8364     10128      8748      7656      4860      3012
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio 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/L	Response Means	Std. Dev.	Pooled Response Means
1	3	0.000	8460.000	166.277	8826.000
2	3	2.420	9192.000	813.791	8826.000
3	3	5.780	8696.000	100.638	8696.000
4	3	14.300	7740.000	562.722	7740.000
5	3	35.600	5020.000	146.642	5020.000
6	3	89.770	3192.000	200.440	3192.000

The Linear Interpolation Estimate: 53.5875 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 53.5602 Standard Deviation: 3.1039
Original Confidence Limits: Lower: 47.3034 Upper: 58.6097
Expanded Confidence Limits: Lower: 40.3909 Upper: 64.1341
Resampling time in Seconds: 0.06 Random_Seed: -354518954