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Data Evaluation Report on the acute toxicity of SXX0665 (JAU6476-desthio) on the Algae, *Scenedesmus subspicatus*

PMRA Submission #: 2004-0843 EPA MRID #: 46246108

Data Requirement:

PMRA DATA CODE	9.8.2
EPA DP Barcode	D303488
OECD Data Point	IIA 8.4.1
EPA MRID	46246108
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: SXX 0665 Technical **Purity:** 93.7%
Common name: JAU6476-desthio
Chemical name: IUPAC: 2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS name: Not reported
CAS No.: 120983-64-4
Synonyms: SXX0665

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 8/18/04

QC Reviewer: Teri Myers, Ph.D.
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Date: 9/15/2005
9/15/05

Secondary Reviewer: Émilie Larivière
HC, PMRA, EAD

Date: 11/3/2005
11/3/05

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

Date Evaluation Completed:

CITATION: Heimbach, F. 1990. Growth Inhibition of Green Algae (*Scenedesmus subspicatus*) by SXX 0665 techn. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Leverkusen, Germany, Laboratory Study No. E 3230401-3, and sponsored by Bayer CropScience, RTP, NC. Experimental start date March 19, 1990 and experimental termination date March 23, 1990. The final report issued June 20, 1990.

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Data Evaluation Report on the acute toxicity of SXX0665 (JAU6476-desthio) on the Algae, *Scenedesmus subspicatus*

PMRA Submission #: 2004-0843 EPA MRID #: 46246108

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to JAU6476-desthio (SXX 0665 Technical) under static conditions at nominal concentrations of 0 (negative control), 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 ppm a.i. (corresponding to 0.01, 0.032, 0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/L). The 0-hour measured concentrations were not detected (control), 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 ppm a.i.. The 96-hour cell density percent inhibitions were 15, 8, 13, 52, 82, 97, 96, 98, 97, and 98% in the 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 25, 11, 12, 60, 79, 94, 94, 96, 96, and 95% in the 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 3, 1, 2, 13, 29, 63, 56, 71, 67, and 68% in the 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9ppm a.i. treatment groups, respectively. Cell density was the most sensitive endpoint tested, based on an EC_{50} of 0.074 ppm a.i.; the NOAEC was <0.011 ppm a.i. and the EC_{05} was 0.011 ppm a.i..

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

Results Synopsis

Test Organism: *Scenedesmus subspicatus*
Test Type: Static

Cell density (day 0 measured):
NOAEC: <0.011 ppm a.i.
LOAEC: 0.011 ppm a.i.
EC_{05}/IC_{05}: 0.011 ppm a.i. 95% C.I.: 0.0039-0.030 ppm a.i.
EC_{50}/IC_{50}: 0.074 ppm a.i. 95% C.I.: 0.045-0.12 ppm a.i.
Slope: 1.97±0.305

Growth rates(day 0 measured):
NOAEC: 0.05 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.241 ppm a.i. 95% C.I.: 0.217-0.256 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve)(day 0 measured):

NOAEC: 0.055 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.077 ppm a.i. 95% C.I.: 0.074-0.081 ppm a.i.
Slope: N/A

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: ISO Guideline ISO/DIS 8692 (Algal Growth Inhibition Test, 1987), OECD Guideline No. 201 "OECD Guideline for Testing of Chemicals, Algal Growth Inhibition Test" (1984), and U.S. EPA FIFRA Guideline 123-2,Tier 2 (and OPPTS Guideline Number 850.5400). The following deviations from U.S. EPA Guideline, §123-2 were noted:

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

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

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice (Chemicals Law (Chem G), dated July 25, 1994, Annex 1, OECD Principles of Good Laboratory Practice (1997), and EPA, FIFRA 40 CFR Part 160 (pp. 1c, 2, and 18).

A. MATERIALS:

1. Test Material JAU6476-desthio(SXX 0665 Technical)

Description: Beige-brown powder

Lot No./Batch No. : 17005/89

Purity: 93.7%

Stability of Compound Under Test Conditions: The 0 hour measured test concentrations were 90-117% of the nominal concentrations (the 96 hour measured concentrations were not determined).

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

Storage conditions of test chemicals: Not reported.

2. Test organism:

Name: *Scenedesmus subspicatus*

EPA requires a nonvascular species: For tier I testing, only one

species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: SAG 86/81
Source: Laboratory culture
Age of inoculum: 3 days old
Method of cultivation: Nutrient Solution (p. 4)

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 96-hour range finding study (pretest) was conducted to determine the definitive nominal concentrations. The concentrations tested were 0.001, 0.01, 0.1, 1.0, and 10 mg/L. The mean cell growth at 96 hours was 367.10, 340.11, 110.32, 11.84, and 9.40 x 10^4 cells/mL in the 0.001, 0.01, 0.1, 1.0, and 10 mg/L treatment groups, respectively, compared to 326.29 x 10 10^4 cells/mL in the control. The percent inhibition for areas under the growth curve were -22, -9, 59, 93, and 95% in the 0.001, 0.01, 0.1, 1.0, and 10 mg/L treatment groups, respectively. The growth rate inhibitions were -2, -1, 19, 58, and 62% in the 0.001, 0.01, 0.1, 1.0, and 10 mg/L treatment groups, respectively.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous	
culturing media and conditions: (same as test or not)	Nutrient solution according to Bringmann and Kuhn (1980, p. 3); the nutrient solution used for testing was Nutrient solution , p. 4.	*EPA recommends two week acclimation period.*
health: (any toxicity observed)	The algae culture was growing exponentially.	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*

Parameter	Details	Remarks *Criteria*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Erlenmeyer flasks 300 mL 150 mL	The test vessels were sealed with cotton wool plugs. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	 Nutrient Solution 7.41-7.96 7.55-8.79 disodium EDTA $NaHCO_3$ N/A	 *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes (p. 4)	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water Sterile Not reported N/A None Not reported Not reported Not reported Not reported Not reported	The dilution water characteristics were not reported. *EPA pH: <u>Skeletonema costatum</u>= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*

Parameter	Details	Remarks *Criteria*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions	
Aeration or agitation	Agitation, 3 rpm	The agitation rate of 3 rpm was less than recommended (100 rpm). *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 6 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicates.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative control), 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 ppm a.i. (corresponding to 0.01, 0.032, 0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/L) Not detected (control), 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 ppm a.i. (Day 0).	92-117% of nominal Table 7, p. 17 *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; 0 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	21.9-23.0°C Continuous 8000 lux (±20%), fluorescent lights.	The light intensity of 8 klux was greater than the recommended 4-5 klux. *EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	Potassium dichromate ($K_2Cr_2O_7$) 0.18, 0.32, 0.56, 1.0, and 1.8 mg/L	This test was conducted on January 2, 1990. The 0-72 hour biomass EC_{50} was 0.60 mg/L. The 0-72 hour growth rate EC_{50} was 1.13 mg/L.
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, growth rate, and area under the growth curve (biomass).	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Counting chamber and microscope.	Photometer used for estimations. *EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water control cell densities at test termination were 322X greater than the dilution water control cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 96-hour cell density percent inhibitions were 15, 8, 13, 52, 82, 97, 96, 98, 97, and 98% in the 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 ppm a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 25, 11, 12, 60, 79, 94, 94, 96, 96, and 95% in the 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 ppm a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 3, 1, 2, 13, 29, 63, 56, 71, 67, and 68% in the 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 ppm a.i. treatment groups, respectively.

Table 3: Effect of Prothioconazole (SXX 0665 Technical) on Algae (*Scenedesmus subspicatus*)

Treatment day 0 measured and nominal concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	108,900	3,221,500	--
0.011 (0.0094)	10,000	77,700	2,734,800	15
0.030 (0.030)	10,000	86,500	2,953,000	8
0.050 (0.052)	10,000	96,700	2,794,600	13
0.085 (0.094)	10,000	47,900	1,532,000	52*
0.17 (0.17)	10,000	80,400	594,100	82*
0.28 (0.30)	10,000	36,400	84,500	97*
0.48 (0.52)	10,000	41,100	129,900	96*
0.87 (0.94)	10,000	33,700	52,000	98*
1.6 (1.7)	10,000	34,400	82,400	97*
2.9 (3.0)	10,000	39,800	62,800	98*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibitions were reviewer-calculated from Table 3, pp. 9-10.
* Significantly different from the control (Tables 6, p. 16).

Table 4: Effect of Prothioconazole (SXX 0665 Technical) on Algae *(Scenedesmus subspicatus)*

Treatment day 0 measured and nominal concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	6.02	--	8588	--
0.011 (0.0094)	10,000	5.85	3	6456	25
0.030 (0.030)	10,000	5.93	1	7664	11
0.050 (0.052)	10,000	5.87	2	7539	12
0.085 (0.094)	10,000	5.24	13	3463	60
0.17 (0.17)	10,000	4.25	29	1824	79
0.28 (0.30)	10,000	2.22	63	523	94
0.48 (0.52)	10,000	2.67	56	544	94
0.87 (0.94)	10,000	1.72	71	382	96
1.6 (1.7)	10,000	2.20	67	350	96
2.9 (3.0)	10,000	1.91	68	408	95
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibitions were reviewer-calculated from Tables 4-5, pp. 12-13.

Table 5: Statistical endpoint values.

Statistical Endpoint[a]	Biomass	Growth rate	Cell density
NOAEC or EC_{05} (ppm a.i.)	0.052	0.052	0.052
EC_{50} (ppm a.i.)	0.076	0.54	Not reported
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	Not reported	Not reported	Not reported
Reference chemical, if used NOAEC EC_{50}	 Not reported 0.60 mg/L	 Not reported 1.13 mg/L	Not reported

[a] Results are based on nominal concentrations.

B. REPORTED STATISTICS:

Statistical Method: The NOAEC and LOAEC values were determined using analysis of variance (Dunnett's-Test). The EC_{50} values for biomass and growth rate (0-96 hours) were calculated using probit analysis by the method of "maximum likelihood". The EC_{50} for cell density was not determined. All statistical calculations were performed using the nominal concentrations.

Cell density:
NOAEC: 0.052 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: Not reported 95% C.I.: Not reported
Slope: N/A

Growth rates:
NOAEC: 0.052 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.54 ppm a.i. 95% C.I.: Not reported
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 0.052 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.076 ppm a.i. 95% C.I.: Not reported
Slope: N/A

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). The NOAEC and LOAEC were determined using ANOVA, followed by Bonferonii's t-test. The analyses described above were conducted using TOXSTAT statistical software and the day 0 measured concentrations were used for all calculations. The EC_{05} and EC_{50} values were determined using the Probit method via Nuthatch statistical software. Growth rates and plant biomass were calculated by the EAD reviewer and were based on day 0 measured values.

Cell density:
NOAEC: <0.011 ppm a.i.
LOAEC: 0.011 ppm a.i.
EC_{05}/IC_{05}: 0.011 ppm a.i. 95% C.I.: 0.0039-0.030 ppm a.i.
EC_{50}/IC_{50}: 0.074 ppm a.i. 95% C.I.: 0.045-0.12 ppm a.i.
Slope: 1.97±0.305

Growth rates (day 0 measured):
NOAEC: 0.05 ppm a.i.

LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.241 ppm a.i. 95% C.I.: 0.217-0.256 ppm a.i.
Slope: N/A

Plant biomass (day 0 measured):

NOAEC: 0.055 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.077 ppm a.i. 95% C.I.: 0.074-0.081 ppm a.i.
Slope: N/A

D. STUDY DEFICIENCIES:

There were no significant study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were not comparable to the study author's because the study author did not analyze cell density data, while this was the only endpoint for which replicate data were provided. Both the study author and the reviewer's results are reported in the Executive Summary and Conclusions sections. Based on these results, cell density was determined to be the most sensitive endpoint, with an EC_{50} of 0.074 ppm a.i..

F. CONCLUSIONS: This study is scientifically sound, fulfills U.S. EPA guideline §123-2, and is classified as ACCEPTABLE. Cell density was the most sensitive endpoint tested, based on an EC_{50} of 0.074 ppm a.i.; the NOAEC was <0.011 ppm a.i. and the EC_{05} was 0.011 ppm a.i..

Cell density (day 0 measured):
NOAEC: <0.011 ppm a.i.
LOAEC: 0.011 ppm a.i.
EC_{05}/IC_{05}: 0.011 ppm a.i. 95% C.I.: 0.0039-0.030 ppm a.i.
EC_{50}/IC_{50}: 0.074 ppm a.i. 95% C.I.: 0.045-0.12 ppm a.i.
Slope: 1.97±0.305

Growth rates (day 0 measured):
NOAEC: 0.05 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.241 ppm a.i. 95% C.I.: 0.217-0.256 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve) (day 0 measured):

NOAEC: 0.055 ppm a.i.
LOAEC: 0.094 ppm a.i.
EC_{50}/IC_{50}: 0.077 ppm a.i. 95% C.I.: 0.074-0.081 ppm a.i.
Slope: N/A

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

III. REFERENCES:

No references were cited.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6108cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	598953.894	66550.433	158.582
Within (Error)	23	9652.167	419.659	
Total	32	608606.061		

Critical F value = 2.32 (0.05,9,23)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 6108cd 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	control	322.167	322.167		
2	0.011	273.667	273.667	3.348	*
3	0.030	295.333	295.333	1.852	
4	0.050	279.333	279.333	2.957	*
5	0.085	153.333	153.333	11.655	*
6	0.17	59.333	59.333	18.145	*
7	0.28	8.667	8.667	21.642	*
8	0.48	13.000	13.000	21.343	*
9	0.87	5.333	5.333	21.872	*
10	1.6	8.333	8.333	21.665	*

Bonferroni T table value = 2.76 (1 Tailed Value, P=0.05, df=23,9)

cell density
File: 6108cd 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	control	6			
2	0.011	3	40.009	12.4	48.500
3	0.030	3	40.009	12.4	26.833
4	0.050	3	40.009	12.4	42.833
5	0.085	3	40.009	12.4	168.833

6	0.17	3	40.009	12.4	262.833
7	0.28	3	40.009	12.4	313.500
8	0.48	3	40.009	12.4	309.167
9	0.87	3	40.009	12.4	316.833
10	1.6	3	40.009	12.4	313.833

cell density
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	322.167	322.167	322.167
2	0.011	3	273.667	273.667	284.500
3	0.030	3	295.333	295.333	284.500
4	0.050	3	279.333	279.333	279.333
5	0.085	3	153.333	153.333	153.333
6	0.17	3	59.333	59.333	59.333
7	0.28	3	8.667	8.667	10.833
8	0.48	3	13.000	13.000	10.833
9	0.87	3	5.333	5.333	6.833
10	1.6	3	8.333	8.333	6.833

cell density
File: 6108cd 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
control	322.167				
0.011	284.500	2.600	*	1.72	k= 1, v=23
0.030	284.500	2.600	*	1.80	k= 2, v=23
0.050	279.333	2.957	*	1.83	k= 3, v=23
0.085	153.333	11.655	*	1.84	k= 4, v=23
0.17	59.333	18.145	*	1.85	k= 5, v=23
0.28	10.833	21.493	*	1.85	k= 6, v=23
0.48	10.833	21.493	*	1.85	k= 7, v=23
0.87	6.833	21.769	*	1.86	k= 8, v=23
1.6	6.833	21.769	*	1.86	k= 9, v=23

s = 20.486
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	0.011	0.0039	0.030	0.22	0.36
EC10	0.017	0.0067	0.041	0.19	0.40
EC25	0.034	0.017	0.068	0.15	0.49
EC50	0.074	0.045	0.12	0.11	0.60

Slope = 1.97 Std.Err. = 0.305

!!!Poor fit: p < 0.001 based on DF= 8.00 25.0

6108CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	322.	332.	-9.93	100.	0.00
0.0110	3.00	274.	315.	-41.4	94.9	5.13
0.0300	3.00	295.	259.	36.1	78.1	21.9
0.0500	3.00	279.	210.	69.4	63.2	36.8
0.0850	3.00	153.	151.	2.64	45.4	54.6
0.170	3.00	59.3	79.4	-20.1	23.9	76.1
0.280	3.00	8.67	42.5	-33.9	12.8	87.2
0.480	3.00	13.0	18.3	-5.33	5.52	94.5
0.870	3.00	5.33	5.86	-0.527	1.76	98.2
1.60	3.00	8.33	1.43	6.90	0.432	99.6
2.90	3.00	6.33	0.286	6.05	0.0861	99.9

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

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: November 3, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Freshwater Algae (green algae, *Scenedesmus subspicatus*)

Heimbach, F. 1990. Growth Inhibition of Green Algae (*Scenedesmus subspicatus*) by SXX 0665 techn. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Leverkusen, Germany, Laboratory Study No. E 3230401-3, and sponsored by Bayer CropScience, RTP, NC. Experimental start date March 19, 1990 and experimental termination date March 23, 1990. The final report issued June 20, 1990.

PMRA DATA CODE: 9.8.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.4.1
EPA MRID: 46246108
EPA Guideline: 123-2 (OPPTS 850.5400)

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to the transformation product JAU6476-desthio (SXX 0665 Technical; purity 93.7%) under static conditions at nominal concentrations of 0 (negative control), 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 mg JAU6476-desthio/L (corresponding to 0.01, 0.032, 0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/L). The 0-hour measured concentrations were not detected (control), 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 mg JAU6476-desthio/L (90-117% of nominal). The 96-hour concentrations were not determined. The study was conducted following ISO-Guideline ISO 8692 (1989) and OECD Guideline 201 and was in compliance with EPA, FIFRA 40 CFR Part 160, and OECD and German Principles of GLP. The 96-hour cell density percent inhibitions were 15, 8, 13, 52, 82, 97, 96, 98, 97, and 98% in the 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 mg JAU6476-desthio/L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 25, 11, 12, 60, 79, 94, 94, 96, 96, and 95% in the 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 mg JAU6476-desthio/L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 3, 1, 2, 13, 29, 63, 56, 71, 67, and 68% in the 0.011, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 mg JAU6476-desthio/L treatment groups, respectively. The NOEC was 0.050 mg a.i./L (day 0 measured) for all endpoints. The EC_{50}/IC_{50}

(and corresponding 95% confidence intervals) for cell density, biomass (area under the growth curve) and growth rate were 0.074 mg JAU6476-desthio/L (0.045-0.12 mg JAU6476-desthio/L), 0.077 mg JAU6476-desthio/L (0.074-0.081 mg JAU6476-desthio/L), and 0.241 mg JAU6476-desthio/L (0.217-0.256 mg JAU6476-desthio/L), respectively.

Results Synopsis (based on Day 0 measured concentrations)

Test Organism: *Scenedesmus subspicatus*
Test Type: Static

Cell density:
NOEC: 0.05 mg JAU6476-desthio/L
LOEC: 0.094 mg JAU6476-desthio/L
EC_{50}/IC_{50}: 0.074 mg JAU6476-desthio/L 95% C.I.: 0.045-0.12 mg JAU6476-desthio/L
Slope: 1.97±0.305

Growth rates:
NOEC: 0.05 mg JAU6476-desthio/L
LOEC: 0.094 mg JAU6476-desthio/L
EC_{50}/IC_{50}: 0.241 mg JAU6476-desthio/L 95% C.I.: 0.217-0.256 mg JAU6476-desthio/L
Slope: N/A

Plant biomass (area under the growth curve):
NOEC: 0.05 mg JAU6476-desthio/L
LOEC: 0.094 mg JAU6476-desthio/L
EC_{50}/IC_{50}: 0.077 mg JAU6476-desthio/L 95% C.I.: 0.074-0.081 mg JAU6476-desthio/L
Slope: N/A

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

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 number and synonym) available from the PMRA Chemistry review.

2. The EAD reviewer verified the biomass and growth rate at 96 hours, based on the cell density and obtained results (means) very close to those reported by the study author. The reviewer used the replicate data for statistical analyses for NOEC and EC_{50}/IC_{50} determination.

3. The EAD verified the statistical analyses for the determation of the NOEC using ANOVA, followed by multiple comparisons. Assumptions of normality and/or homogeneity of variances were not met for cell density and for growth rate and transformations were not successful. Non-parametric ANOVA on ranks were run and did not reveal significant differences until the 0.28 mg JAU6476-desthio/L treatment level. This is undoubtedly due to the low power of the non-parametric test. Based on inspection of the data and of the variability, the EAD reviewer agrees with the study author that the NOEC is 0.05 mg JAU6476-desthio/L for these two endpoints. The EPA reviewer reported a NOEC of <0.011 mg JAU6476-desthio/L. The ANOVA for biomass indicated a significant difference at the lowest treatment level, but not at the next two higher treatment levels. Upon plotting the data, it was determined that the NOEC should be set at 0.05 mg JAU6476-desthio/L.

4. The EAD reviewer verified the 96-hour EC_{50}/IC_{50} for cumulative biomass as well as growth rate with linear interpolation (ICp; US EPA, 1993) and using nominal as well as day 0 measured concentrations. The difference between the nominal and day 0 results is extremely small. Results of the EAD reviewer for biomass and growth rate (day 0 measured concentrations) will be reported in the EAD Executive Summary. The EAD reviewer agrees with the 96-hour EC_{50}/IC_{50} reported by the EPA reviewer.

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

Statistical verification of the EAD reviewer

Cell density

Density ranks

One Way Analysis of VarianceTuesday, November 01, 2005, 10:51:39

Data source: Data 1 in Notebook

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Tuesday, November 01, 2005, 10:51:39

Data source: Data 1 in Notebook

Group	N	Missing	Median	25%	75%
control	6	0	333.500	306.000	335.000
0.0110	3	0	271.000	270.250	277.750
0.0300	3	0	299.000	284.000	305.750
0.0500	3	0	275.000	262.250	297.500
0.0850	3	0	152.000	136.250	170.750
0.170	3	0	41.000	31.250	92.000
0.280	3	0	8.000	5.750	11.750
0.480	3	0	13.000	11.500	14.500
0.870	3	0	6.000	3.750	6.750
1.600	3	0	9.000	6.750	9.750
2.900	3	0	6.000	4.500	8.250

H = 32.733 with 10 degrees of freedom. (P = <0.001)

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

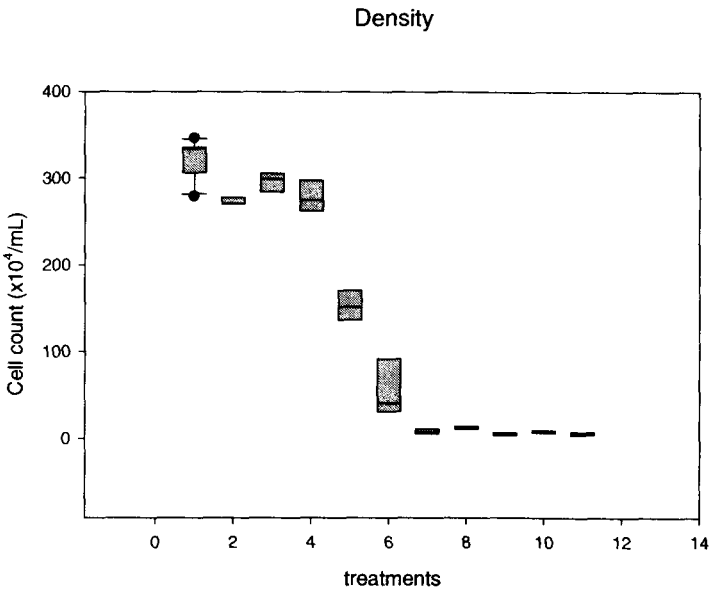
To isolate the group or groups that differ from the others use a multiple comparison procedure.

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

Comparison	Diff of Ranks	Q	P<0.05
0.87 vs control	28.250	3.792	Yes
2.9 vs control	27.083	3.635	Yes
0.28 vs control	24.417	3.277	Yes
1.6 vs control	24.083	3.233	Yes
0.48 vs control	19.083	2.562	No

0.17 vs control	15.583	2.092	Do Not Test
0.085 vs control	12.583	1.689	Do Not Test
0.011 vs control	7.583	1.018	Do Not Test
0.05 vs control	6.917	0.928	Do Not Test
0.03 vs control	3.417	0.459	Do Not Test

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



Biomass

Biomass

One Way Analysis of VarianceTuesday, November 01, 2005, 11:31:33

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.015)

Equal Variance Test: Passed (P = 0.053)

Group Name	N	Missing	Mean	Std Dev	SEM
control	6	0	8594.000	625.540	255.376
0.0110	3	0	6456.000	491.414	283.718
0.0300	3	0	7668.000	174.310	100.638
0.0500	3	0	7548.000	847.424	489.261
0.0850	3	0	3476.000	630.466	364.000
0.170	3	0	1820.000	973.579	562.096
0.280	3	0	532.000	90.067	52.000
0.480	3	0	536.000	99.920	57.689
0.870	3	0	380.000	172.372	99.519
1.600	3	0	344.000	66.091	38.158
2.900	3	0	392.000	61.579	35.553

Source of Variation	DF	SS	MS	F	P
Between Groups	10	427788596.000	42778859.600	158.696	<0.001
Residual	25	6739128.000	269565.120		
Total	35	434527724.000			

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

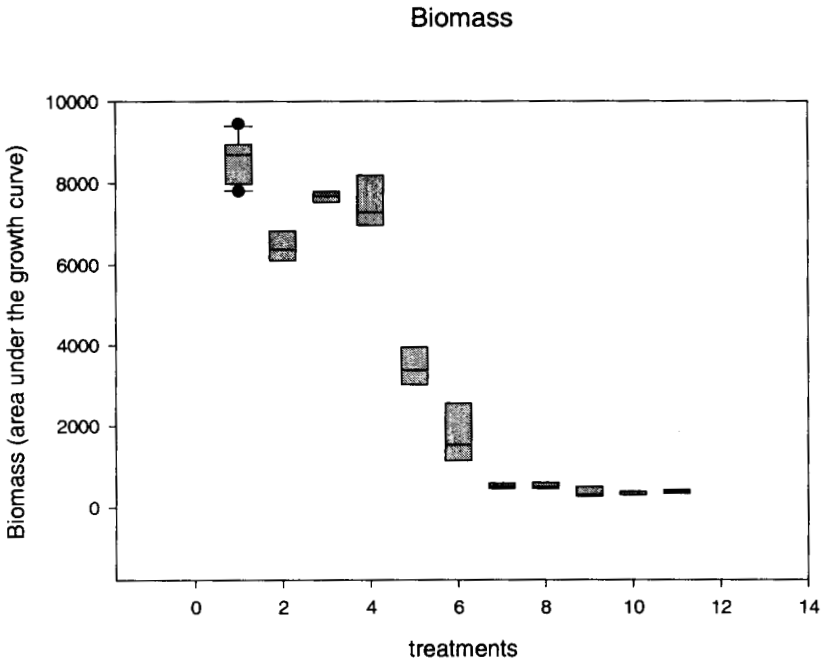
Power of performed test with alpha = 0.050: 1.000

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

Comparisons for factor: treatments

Comparison	Diff of Means	t	P	P<0.050
control vs. 1.600	8250.000	22.472	<0.001	Yes
control vs. 0.870	8214.000	22.374	<0.001	Yes
control vs. 2.900	8202.000	22.341	<0.001	Yes
control vs. 0.280	8062.000	21.960	<0.001	Yes
control vs. 0.480	8058.000	21.949	<0.001	Yes
control vs. 0.170	6774.000	18.451	<0.001	Yes
control vs. 0.085	5118.000	13.941	<0.001	Yes
control vs. 0.011	2138.000	5.824	<0.001	Yes
control vs. 0.050	1046.000	2.849	0.086	No
control vs. 0.030	926.000	2.522	0.184	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.



Conc. ID		1	2	3	4	5	6	7	8	9	10	11
Conc. Tested		0	0.011	0.03	0.050	0.085	0.17	0.28	0.48	0.87	1.6	2.9
Response	1	8892	6372	7836	7284	2904	1536	444	456	576	312	324
Response	2	8496	6984	7680	6864	4152	2904	528	648	252	420	408
Response	3	7980	6012	7488	8496	3372	1020	624	504	312	300	444
Response	4	7800										
Response	5	8952										
Response	6	9444										

*** Inhibition Concentration Percentage Estimate ***

Toxicant/Effluent: JAU6476-desthio Biomass day 0
Test Start Date: Test Ending Date:
Test Species: Scenedesmus subspicatus
Test Duration: 96 hous
DATA FILE: ScebioD0.icp
OUTPUT FILE: ScebioD0.i50

Conc. ID	Number Replicates	Concentration mg desthio/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	8594.000	625.540	8594.000
2	3	0.011	6456.000	491.414	7224.000
3	3	0.030	7668.000	174.310	7224.000
4	3	0.050	7548.000	847.424	7224.000
5	3	0.085	3476.000	630.466	3476.000
6	3	0.170	1820.000	973.579	1820.000
7	3	0.280	532.000	90.067	534.000
8	3	0.480	536.000	99.920	534.000
9	3	0.870	380.000	172.372	380.000
10	3	1.600	344.000	66.091	368.000
11	3	2.900	392.000	61.579	368.000

The Linear Interpolation Estimate: 0.0773 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 0.0768 Standard Deviation: 0.0024
Original Confidence Limits: Lower: 0.0735 Upper: 0.0806
Expanded Confidence Limits: Lower: 0.0723 Upper: 0.0816
Resampling time in Seconds: 0.00 Random_Seed: -1214778

Growth Rate

Growth rate ranks

One Way Analysis of Variance Tuesday, November 01, 2005, 11:33:55

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.032)

Equal Variance Test: Failed (P = 0.007)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Tuesday, November 01, 2005, 11:33:55

Data source: Data 1 in Notebook

Group	N	Missing	Median		25%	75%
control	6	0	0.0605	0.0596	0.0606	
0.0110	3	0	0.0584	0.0583	0.0586	
0.0300	3	0	0.0594	0.0588	0.0596	
0.0500	3	0	0.0585	0.0580	0.0593	
0.0850	3	0	0.0523	0.0512	0.0535	
0.170	3	0	0.0387	0.0357	0.0463	
0.280	3	0	0.0217	0.0180	0.0255	
0.480	3	0	0.0267	0.0254	0.0278	
0.870	3	0	0.0187	0.0132	0.0199	
1.600	3	0	0.0229	0.0197	0.0237	
2.900	3	0	0.0187	0.0155	0.0218	

H = 32.733 with 10 degrees of freedom. (P = <0.001)

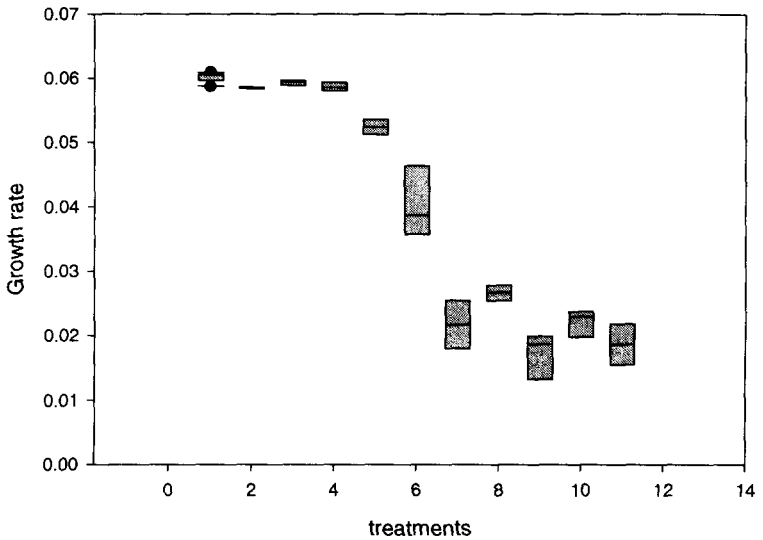
The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

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

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

Comparison	Diff of Ranks	Q	P<0.05
0.87 vs control	28.250	3.792	Yes
2.9 vs control	27.083	3.635	Yes
0.28 vs control	24.417	3.277	Yes
1.6 vs control	24.083	3.233	Yes
0.48 vs control	19.083	2.562	No
0.17 vs control	15.583	2.092	Do Not Test
0.085 vs control	12.583	1.689	Do Not Test
0.011 vs control	7.583	1.018	Do Not Test
0.05 vs control	6.917	0.928	Do Not Test
0.03 vs control	3.417	0.459	Do Not Test

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




```
Conc. ID          1
  2     3     4
5     6     7     8
 9    10    11
-----------------
-----------------
-----------------
----------------
Conc. Tested
00.011 0.03
0.050.085 0.17
0.28 0.48 0.87
1.6  2.9
-----------------
-----------------
-----------------
----------------
Response  1
0.0605330.0586960
.0596890.0578430.
0507830.0386830.0
216610.0249780.02
0270.0228880.0186
64
```

```
Response  2
0.0605640.0583550.059380.0585080.0539180.0488680.0167650.0282090.0186640.02398
50.022888
Response  3
0.0596210.0583170.0585680.0595870.0523320.034710.0267180.0267180.0114440.01866
40.014441
Response  4 0.058658
Response  5 0.060501
Response  6 0.0609
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-desthio  Growth rate day 0
Test Start Date:    Test Ending Date:
Test Species: Scenedesmus subspicatus
Test Duration:        96 hours
DATA FILE: Scegrod0.icp
OUTPUT FILE: scegrod0.i50
---------------------------------------------------------------------
```

Conc. ID	Number Replicates	Concentration mg desthio/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.060	0.001	0.060
2	3	0.011	0.058	0.000	0.059
3	3	0.030	0.059	0.001	0.059
4	3	0.050	0.059	0.001	0.059
5	3	0.085	0.052	0.002	0.052
6	3	0.170	0.041	0.007	0.041
7	3	0.280	0.022	0.005	0.024
8	3	0.480	0.027	0.002	0.024
9	3	0.870	0.017	0.005	0.019
10	3	1.600	0.022	0.003	0.019
11	3	2.900	0.019	0.004	0.019

```
---------------------------------------------------------------------
The Linear Interpolation Estimate:     0.2409   Entered P Value: 50
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   0.2389 Standard Deviation:     0.0114
Original Confidence Limits:   Lower:     0.2173 Upper:     0.2556
Expanded Confidence Limits:   Lower:     0.2102 Upper:     0.2600
Resampling time in Seconds:     0.00  Random_Seed: -1512070394
```