Data Evaluation Report on the acute toxicity of Prothioconazole Metabolite (JAU6476-S-Methyl) on the Algae, *Selenastrum capricornutum*

PMRA Submission #: 2004-0843 EPA MRID #: 46246107

Data Requirement:

PMRA DATA CODE	9.8.2
EPA DP Barcode	D303488
OECD Data Point	IIA 8.4.1
EPA MRID	46246107
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: JAU6476-S-Methyl **Purity:** 98.6%

Common name: Prothioconazole metabolite

Chemical name: IUPAC: 1H-1,2,4-triazole-1-ethanol, a-(1-chlorocyclopropyl)-a-[(2-chlorophenyl)mehtyl]-5-(methylthio)-

CAS name: Not reported

CAS No.:178928-71-7

Synonyms: Metabolite of JAU 6476; WAK7861

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Date: 11/2/2005
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Company Code: BCZ
Active Code: PRB
Use Site Category: 7, 13, 14
EPA PC Code: 113961

Date Evaluation Completed:

CITATION: Dorgerloh, M and H. Sommer. 2001. JAU6476-S-Methyl Influence on the Growth of the Green Alga, *Selenastrum capricornutum*. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany, Laboratory Study No. E 3232061-7, and sponsored by Bayer CropScience, RTP, NC. Experimental start date June 21, 2001 and experimental termination date July 3, 2001. The final report issued July 20, 2001.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Prothioconazole metabolite (JAU 6476-S-Methyl) under static conditions at nominal concentrations of 1.54, 3.09, 6.16, 12.3, 24.7, 49.3, and 98.6 mg a.s./L. The 0-hour measured concentrations were 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L; the 0-hour measured concentrations were <70% of nominal for all test concentrations and the poor recovery of the test material was not discussed. The 96-hour cell density percent inhibitions were 18.91, 31.09, 52.01, 70.80, 79.23, 81.09, and 87.56% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 16.5, 29.6, 50.5, 69.4, 76.7, 79.2, and 85.3% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 3.2, 6.2, 12.9, 21.8, 28.1, 29.7, and 37.4% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively. The NOAEC was <1.03 mg a.s./L for all endpoints. Cell density was the most sensitive endpoint tested (and the only endpoint for which replicate data were provided), based on an EC_{50} of 2.8 mg a.s./L.

The study is scientifically sound, but it does not satisfy the U.S. EPA Guideline §123-2 for an aquatic nonvascular plant study with *Selenastrum capricornutum* because the analytical recovery of the test material at test initiation was less than 70% of nominal for all test levels and this issue was not addressed in the study report. As a result, this study is classified as Supplemental. Results from this study may be useful for future risk assessments.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell density; reviewer-reported (96-hour):
NOAEC: <1.03 mg a.i./L
LOAEC: 1.03 mg a.i./L
EC_{05}/IC_{05}: 0.092 mg a.s./L 95% C.I.: 0.033-0.26 mg a.s./L
EC_{50}/IC_{50}: 2.8 mg a.s./L 95% C.I.: 1.8-4.2 mg a.s./L
Slope: 1.11±0.112

Growth rates; study author-reported (72-hour):
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 47.4 mg a.s./L 95% C.I.: Not reported
Slope: N/A

Plant biomass (area under the growth curve); study author-reported (72-hour):
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 3.77 mg a.s./L 95% C.I.: Not reported
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: EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218, 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 measured test concentrations were all <70% of nominal at 0 hours, and three concentrations (nominal 12.3, 24.7, and 98.6 mg a.i./L) were <70% of nominal at 96 hours. No discussion was provided regarding this deviation.

2. The negative control medium was apparently not analyzed for active ingredient.

3. The agitation rate of 3 rpm was less than recommended (100 rpm).

4. The light intensity of 8 klux was greater than the recommended 4-5 klux.

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, and the OECD Principles of Good Laboratory Practice (1997), (pp. 1c and 2).

A. MATERIALS:

1. Test Material Prothioconazole metabolite (JAU 6476-S-Methyl)

Description: Beige solid

Lot No./Batch No. : HUPP0658-MP

Purity: 98.6%

Stability of Compound Under Test Conditions: The 0 hour measured test concentrations were 31-67% of the nominal concentrations and the 96 hour measured test concentrations were 48-84% of the nominal concentrations.

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

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Name: *Selenastrum capricornutum*

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 61.81
Source: Collection of Algal Cultures, Institute for Plant Physiology, University of Gottingen, Gottingen, Germany.
Age of inoculum: 3 days old
Method of cultivation: Nutrient Solution 2 (p. 9)

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range finding study was reported.

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 1 (p. 8); this culture nutrient solution was modified for testing (Nutrient solution 2).	*EPA recommends two week acclimation period.*
health: (any toxicity observed)	Not reported.	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	

Parameter	Details	Remarks *Criteria*
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 2 7.98-8.42 8.85-10.02 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. 9)	
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: Skeletonema costatum = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions	

Parameter	Details	Remarks *Criteria*
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), 1.54, 3.09, 6.16, 12.3, 24.7, 49.3, and 98.6 mg a.s./L (corresponding to 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg/L) Day 0: 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L. Day 4: 1.19, 2.61, 5.09, 6.71, 11.9, 35.9, 51.1 mg a.s./L	The control group was not measured for active ingredient. The quantities of test item found at the beginning of the test in reference to the nominal concentrations were 31-67% (average 47%). The quantities found at the end (day 4) were 48-84% (average 67%). *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 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	23±2°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.*

Parameter	Details	Remarks *Criteria*
Reference chemical {if used) name: concentrations:	Potassium dichromate ($K_2Cr_2O_7$) 0.10, 0.18, 0.32, 0.56, 1.00, and 1.80 mg/L	This test was conducted on March 9, 2001. The 0-72 hour biomass EC_{50} was 1.10 mg/L. The 0-72 hour growth rate EC_{50} was 2.35 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	

Parameters	Details	Remarks/Criteria
Indicate whether there was exponential growth in the control	Yes, dilution water control cell densities at test termination were 221X greater than the dilution water control cell densities at test initiation.	*EPA requires control cell count at termination to be ~~2~~X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 96-hour cell density percent inhibitions were 18.91, 31.09, 52.01, 70.80, 79.23, 81.09, and 87.56% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 16.5, 29.6, 50.5, 69.4, 76.7, 79.2, and 85.3% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 3.2, 6.2, 12.9, 21.8, 28.1, 29.7, and 37.4% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively.

Table 3: Effect of Prothioconazole Metabolite (JAU6476-S-Methyl) on Algae (*Selenastrum capricornutum*)

Treatment day 0 measured and nominal concentrations [a] (mg a.s./L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	36,000	2,214,000	--
1.03 (1.54)	10,000	36,600	1,864,000	18.91*
1.60 (3.09)	10,000	34,700	1,584,000	31.09*
2.84 (6.16)	10,000	30,400	1,103,000	52.01*
4.91 (12.3)	10,000	29,100	671,000	70.80*
8.74 (24.7)	10,000	29,700	477,300	79.23*
15.4 (49.3)	10,000	29,700	434,700	81.09*
55.5 (98.6)	10,000	19,800	286,000	87.56*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.

[b] The 96 hour cell densities and percent inhibitions were obtained from Table 6c, p. 22.
* Significantly different from the control (Dunnett's test).

Table 4: Effect of Prothioconazole Metabolite (JAU6476-S-Methyl) on Algae *(Selenastrum capricornutum)*

Treatment day 0 measured and Concentrations [a] (mg a.s./L)	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	1.37	--	4943	--
1.03 (1.54)	10,000	1.33	3.2*	4129	16.5*
1.60 (3.09)	10,000	1.28	6.2*	3481	29.6*
2.84 (6.16)	10,000	1.19	12.9*	2447	50.5*
4.91 (12.3)	10,000	1.07	21.8*	1513	69.4*
8.74 (24.7)	10,000	0.98	28.1*	1154	76.7*
15.4 (49.3)	10,000	0.96	29.7*	1028	79.2*
55.5 (98.6)	10,000	0.86	37.4*	729	85.3*
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The values were based on 0-96 hour data. The statistical analyses used 0-72 hour data.
* Significantly different from the control (Tables 6a-6b, pp. 20-21).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density
NOAEC or EC_{05} (mg a.s./L)	<1.03	<1.03	<1.03
EC_{50} (mg a.s./L)	3.77	47.4	2.69
IC_{50} or EC_{50} (mg a.s./L) (95% C.I.)	Not reported	Not reported	Not reported
Reference chemical, if used NOAEC EC_{50}	 Not reported 1.10 mg/L	 Not reported 2.35 mg/L	Not reported

[a] The biomass and growth rate statistics based on 0-72 hour data.

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 (0-72 hours) were calculated using probit analysis. The EC_{50} for cell density was based on 96 hour cell densities. The statistical analyses were conducted using the commercial software (Ratte, 1993-1998). All statistical calculations were performed using the initial (Day 0) measured concentrations.

Cell density:
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 2.69 mg a.s./L 95% C.I.: Not reported
Slope: N/A

Growth rates:
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 47.4 mg a.s./L 95% C.I.: Not reported
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 3.77 mg a.s./L 95% C.I.: Not reported
Slope: N/A

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

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 William's multiple comparison 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.

Cell density:
NOAEC: <1.03 mg a.i./L
LOAEC: 1.03 mg a.i./L
EC_{05}/IC_{05}: 0.092 mg a.s./L 95% C.I.: 0.033-0.26 mg a.s./L
EC_{50}/IC_{50}: 2.8 mg a.s./L 95% C.I.: 1.8-4.2 mg a.s./L
Slope: 1.11±0.112

D. STUDY DEFICIENCIES:

The measured initial test solution concentrations were (in some cases) significantly lower than 70% of nominal. This issue was not addressed by the study authors. Failure to discuss this deviation affected the acceptability of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the NOAEC and EC_{50} values for cell density were similar to the study authors'. Because the reviewer's estimate was associated with a 95% confidence interval and slope value, it is reported in the Executive Summary and Conclusions sections; the study authors' conclusions are reported for growth rate and biomass (endpoints for which replicate data were not provided).

F. CONCLUSIONS: The study is scientifically sound, but it does not satisfy the U.S. EPA Guideline §123-2 for an aquatic nonvascular plant study with *Selenastrum capricornutum* because the analytical recovery of the test material at test initiation was less than 70% of nominal for all test levels and this issue was not addressed in the study report. As a result, this study is classified as Supplemental. Cell density was the most sensitive endpoint tested (and the only endpoint for which replicate data were provided), based on an EC_{50} of 2.8 mg a.s./L.

Cell density; reviewer-reported:
NOAEC: <1.03 mg a.i./L
LOAEC: 1.03 mg a.i./L
EC_{05}/IC_{05}: 0.092 mg a.s./L 95% C.I.: 0.033-0.26 mg a.s./L
EC_{50}/IC_{50}: 2.8 mg a.s./L 95% C.I.: 1.8-4.2 mg a.s./L
Slope: 1.11±0.112

Growth rates; study author-reported:
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 47.4 mg a.s./L 95% C.I.: Not reported
Slope: N/A

Plant biomass (area under the growth curve); study author-reported:
NOAEC: <1.03 mg a.s./L
LOAEC: 1.03 mg a.s./L
EC_{50}/IC_{50}: 3.77 mg a.s./L 95% C.I.: Not reported
Slope: N/A

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

III. REFERENCES:

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Litchfield, J.F. and Wilcoxon, F.A. (1949). A simplified method of evaluating dose-effect experiments. J. Pharmacol. 31: 99-113

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U.S. EPA-FIFRA,.Subdivision N, § 123-2 "Growth and Reproduction of Aquatic Plants, Tier 2"

U.S. EPA-Guideline 540/9-86-134 (Environmental Protection Agency, Hazard Evaluation Division, Standard Evaluation Procedure-Non-Target Plants Growth and Reproduction of Aquatic Plants, Tiers 1 and 2, June 1986).

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6107cd Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	146696.760	20956.680	218.060
Within (Error)	19	1826.000	96.105	
Total	26	148522.760		

Critical F value = 2.54 (0.05,7,19)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 6107cd 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	control	221.733	221.733		
2	1.03	186.367	186.367	5.102	*
3	1.6	158.367	158.367	9.141	*
4	2.84	110.300	110.300	16.075	*
5	4.91	67.100	67.100	22.307	*
6	8.74	47.733	47.733	25.101	*
7	15.4	43.467	43.467	25.717	*
8	55.5	28.600	28.600	27.861	*

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

cell density
File: 6107cd 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	control	6			
2	1.03	3	18.696	8.4	35.367
3	1.6	3	18.696	8.4	63.367
4	2.84	3	18.696	8.4	111.433
5	4.91	3	18.696	8.4	154.633
6	8.74	3	18.696	8.4	174.000
7	15.4	3	18.696	8.4	178.267
8	55.5	3	18.696	8.4	193.133

cell density

File: 6107cd Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	221.733	221.733	221.733
2	1.03	3	186.367	186.367	186.367
3	1.6	3	158.367	158.367	158.367
4	2.84	3	110.300	110.300	110.300
5	4.91	3	67.100	67.100	67.100
6	8.74	3	47.733	47.733	47.733
7	15.4	3	43.467	43.467	43.467
8	55.5	3	28.600	28.600	28.600

cell density
File: 6107cd 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
control	221.733				
1.03	186.367	5.102	*	1.73	k= 1, v=19
1.6	158.367	9.141	*	1.81	k= 2, v=19
2.84	110.300	16.075	*	1.84	k= 3, v=19
4.91	67.100	22.307	*	1.85	k= 4, v=19
8.74	47.733	25.101	*	1.86	k= 5, v=19
15.4	43.467	25.716	*	1.87	k= 6, v=19
55.5	28.600	27.861	*	1.87	k= 7, v=19

s = 9.803
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	0.092	0.033	0.26	0.21	0.36
EC10	0.20	0.082	0.47	0.18	0.42
EC25	0.69	0.36	1.3	0.13	0.53
EC50	2.8	1.8	4.2	0.086	0.66

Slope = 1.11 Std.Err. = 0.112

!!!Poor fit: p < 0.001 based on DF= 5.00 19.0

6107CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	222.	229.	-7.28	100.	0.00
1.03	3.00	186.	157.	29.8	68.4	31.6
1.60	3.00	158.	138.	19.9	60.5	39.5

2.84	3.00	110.	113.	-3.07	49.5	50.5
4.91	3.00	67.1	89.5	-22.4	39.1	60.9
8.74	3.00	47.7	66.2	-18.4	28.9	71.1
15.4	3.00	43.5	46.5	-3.05	20.3	79.7
55.5	3.00	28.6	16.8	11.8	7.34	92.7

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

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

!!!Warning: EC25 not bracketed by doses evaluated.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: November 2, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Freshwater Algae (*Selenastrum capricornutum*)

Dorgerloh, M and H. Sommer. 2001. JAU6476-S-Methyl Influence on the Growth of the Green Alga, *Selenastrum capricornutum*. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany, Laboratory Study No. E 3232061-7, and sponsored by Bayer CropScience, RTP, NC. Experimental start date June 21, 2001 and experimental termination date July 3, 2001. The final report issued July 20, 2001.

PMRA DATA CODE: 9.8.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.4.1
EPA MRID: 46246107
EPA Guideline: 123-2 (OPPTS 850.5400)

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Prothioconazole metabolite (JAU 6476-S-Methyl) under static conditions at nominal concentrations of 1.54, 3.09, 6.16, 12.3, 24.7, 49.3, and 98.6 mg JAU6476-S-methyl/L. The 0-hour measured concentrations were 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg JAU6476-S-methyl/L (31-67% of nominal), while the 96-hour measured concentrations were 1.19, 2.61, 5.09, 6.71, 11.9, 35.9, and 51.1 mg JAU6476-S-methyl/L (48-84% of nominal). The poor recovery of the test material was not discussed. The study followed EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218, and U.S. EPA-FIFRA Guideline 123-2,Tier 2 and was conducted in compliance with German and OECD Principles of GLP. The 96-hour cell density percent inhibitions were 18.91, 31.09, 52.01, 70.80, 79.23, 81.09, and 87.56% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg JAU6476-S-methyl/L treatment groups, respectively (day 0 measured concentrations). The area under the growth curve (0 to 96 hours) percent inhibitions were 16.5, 29.6, 50.5, 69.4, 76.7, 79.2, and 85.3% in the 1.03, 1.60, 2.84, 4.91, 8.74, 15.4, and 55.5 mg JAU6476-S-methyl/L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 3.2, 6.2, 12.9, 21.8, 28.1, 29.7, and 37.4% in the 1.03, 1.60, 2.84, 4.91,

8.74, 15.4, and 55.5 mg JAU6476-S-methyl/L treatment groups, respectively. The NOEC was <1.03 mg JAU6476-S-methyl/L for all endpoints. The EC_{50}/IC_{50}s (and corresponding 95% confidence intervals) for cell density (96 hours), area under the growth curve (72 hours) and growth rate (72 hours) were 2.8 mg JAU6476-S-methyl/L (1.8-4.2 mg JAU6476-S-methyl/L), 3.0 mg JAU6476-S-methyl/L (2.70-3.40 mg JAU6476-S-methyl/L) and >55.5 mg JAU6476-S-methyl/L, respectively.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell density (96 hours); EPA reviewer-reported:
NOEC: <1.03 JAU6476-S-methyl/L
LOEC: 1.03 JAU6476-S-methyl/L
EC_{50}/IC_{50}: 2.8 JAU6476-S-methyl/L 95% C.I.: 1.8-4.2 JAU6476-S-methyl/L
Slope: 1.11±0.112

Growth rates (72 hours); EAD reviewer-reported:
NOEC: <1.03 JAU6476-S-methyl/L
LOEC: 1.03 JAU6476-S-methyl/L
EC_{50}/IC_{50}: >55.5 mg JAU6476-S-methyl/L 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve)(72 hours); EAD reviewer-reported:
NOEC: <1.03 JAU6476-S-methyl/L
LOEC: 1.03 JAU6476-S-methyl/L
EC_{50}/IC_{50}: 3.0 JAU6476-S-methyl/L 95% C.I.: 2.70-3.40 mg JAU6476-S-methyl/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 other sources such as the PMRA Chemistry review.

2. The EAD reviewer finds that using the day 0 measured concentrations is appropriate in this study,

as they are a conservative estimate of exposure. Uncertainty exists as to what the exposure levels were, as day 0 and day 4 measurements were 31-67 and 48-84% of nominal concentrations, respectively. Six out of 7 treatment levels had measured concentrations higher on Day 4 were higher than day 0 (3 were significantly higher). This seems to indicate a problem with analysis of the chemical. To air on the conservative side, day 0 concentrations will be used, as they are generally lower than the day 4 values.

3. The EAD reviewer agrees with the conclusions of the study author and the EPA reviewer regarding the NOECs. The EAD reviewer did not feel that redoing the statistical analyses was necessary. The EAD reviewer verified data for the biomass and growth rate at 72 hours, based on the cell density data and obtained results very close to those reported by the study author. The EC_{50}/IC_{50} was verified by the EAD reviewer for cell density (96-hour) and for area under the growth curve (72-hour) using linear interpolation (ICp, US EPA 1993). The 72-hour EC_{50}/IC_{50} was not calculated for growth rate, as 50% inhibition was not reached at the highest treatment level at either 72 or 96 hours. The highest inhibition observed was 45.9% compared to the control after 72 hours. The 96-hour EC_{50}/IC_{50} for cell density was very similar to the reported value of the EPA reviewer. The values of the EPA reviewer are considered acceptable. The 72-hour EC_{50}/IC_{50} for biomass was similar to that reported by the study author; however, the values of the EAD reviewer have associated confidence intervals. The values of the EAD reviewer will therefore be reported in the EAD Executive Summary.

Study Acceptability: The study is scientifically sound, but the analytical recovery of the test material at test initiation was less than 70% of nominal for all test levels and this issue was not addressed in the study report. Recoveries after 4 days of exposure were higher than those at test initiation in 6 out of the 7 treatment levels, which indicates a possible problem with analysis of the chemical. As a result, it is difficult to determine an estimate of exposure with certainty. Results from this study may still be useful for future risk assessments.

Cell density

```
Conc. ID        1    2    3    4    5    6    7    8
-------------------------------------------------------
Conc. Tested    0 1.03  1.6 2.84 4.91 8.74 15.4 55.5
-------------------------------------------------------
Response  1 218.5176.6149.6 91.2 65.7 53.4 42.3   31
Response  2 233.8189.4171.2113.5 71.9 45.4 45.2 25.8
Response  3 231.7193.1154.3126.2 63.7 44.4 42.9   29
Response  4   224
Response  5 221.3
Response  6 201.1
-------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-S-methyl Day 0 cell density-72-h
Test Start Date:    Test Ending Date:
Test Species: Selenastrum capricornutum
Test Duration:        96 hours
DATA FILE: SeldenS0.icp
OUTPUT FILE: SeldenS0.i50
```

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	221.733	11.717	221.733
2	3	1.030	186.367	8.658	186.367
3	3	1.600	158.367	11.360	158.367
4	3	2.840	110.300	17.718	110.300
5	3	4.910	67.100	4.276	67.100
6	3	8.740	47.733	4.933	47.733
7	3	15.400	43.467	1.531	43.467
8	3	55.500	28.600	2.623	28.600

```
The Linear Interpolation Estimate:     2.8254   Entered P Value: 50
-------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   2.8788 Standard Deviation:     0.2302
Original Confidence Limits:   Lower:     2.5077 Upper:     3.3383
Expanded Confidence Limits:   Lower:     2.4124 Upper:     3.4922
Resampling time in Seconds:     0.00  Random_Seed: 2137316380

Conc. ID        1    2    3    4    5    6    7    8
-------------------------------------------------------
Conc. Tested    0 1.03  1.6 2.84 4.91 8.74 15.4 55.5
-------------------------------------------------------
Response  1 218.5176.6149.6 91.2 65.7 53.4 42.3   31
Response  2 233.8189.4171.2113.5 71.9 45.4 45.2 25.8
Response  3 231.7193.1154.3126.2 63.7 44.4 42.9   29
Response  4   224
Response  5 221.3
Response  6 201.1
-------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-S-methyl Day 0 cell density-72-h
```

```
Test Start Date:     Test Ending Date:
Test Species: Selenastrum capricornutum
Test Duration:         96 hours
DATA FILE: SeldenS0.icp
OUTPUT FILE: SeldenS0.i05
```

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	221.733	11.717	221.733
2	3	1.030	186.367	8.658	186.367
3	3	1.600	158.367	11.360	158.367
4	3	2.840	110.300	17.718	110.300
5	3	4.910	67.100	4.276	67.100
6	3	8.740	47.733	4.933	47.733
7	3	15.400	43.467	1.531	43.467
8	3	55.500	28.600	2.623	28.600

```
The Linear Interpolation Estimate:      0.3229   Entered P Value:  5
Number of Resamplings:   80
The Bootstrap Estimates Mean:    0.3370 Standard Deviation:      0.0542
Original Confidence Limits:   Lower:      0.2492 Upper:      0.4347
Expanded Confidence Limits:   Lower:      0.2271 Upper:      0.4682
Resampling time in Seconds:      0.00  Random_Seed: 189194636
```

Plant Biomass

```
Conc. ID        1    2    3    4    5    6    7    8
-------------------------------------------------------
Conc. Tested    0 1.03  1.6 2.84 4.91 8.74 15.4 55.5
-------------------------------------------------------
Response  1 1448.41130.4955.2640.8  462  420381.6333.6
Response  2 1425.61179.6945.6710.4481.2382.8382.8278.4
Response  3 1406.41174.81040.4  774  462298.4  330  228
Response  4 1329.6
Response  5 1420.8
Response  6 1250.4
-------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-S-methyl  Day 0  biomass (72-h)
Test Start Date:     Test Ending Date:
Test Species: Selenastrum capricornutum
Test Duration:         96 hours
DATA FILE: SelbioS0.icp
OUTPUT FILE: SelbioS0.i50
```

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	1380.200	75.440	1380.200
2	3	1.030	1161.600	27.126	1161.600

3	3	1.600	980.400	52.183	980.400
4	3	2.840	708.400	66.623	708.400
5	3	4.910	468.400	11.085	468.400
6	3	8.740	367.067	62.308	367.067
7	3	15.400	364.800	30.144	364.800
8	3	55.500	280.000	52.818	280.000

The Linear Interpolation Estimate: 2.9978 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 3.0312 Standard Deviation: 0.2093
Original Confidence Limits: Lower: 2.6988 Upper: 3.4002
Expanded Confidence Limits: Lower: 2.6091 Upper: 3.5209
Resampling time in Seconds: 0.00 Random_Seed: -5015444

Conc. ID	1	2	3	4	5	6	7	8
Conc. Tested	0	1.03	1.6	2.84	4.91	8.74	15.4	55.5
Response 1	1448.4	1130.4	955.2	640.8	462	420	381.6	333.6
Response 2	1425.6	1179.6	945.6	710.4	481.2	382	8382.8	278.4
Response 3	1406.4	1174.8	1040.4	774	462	298.4	330	228
Response 4	1329.6							
Response 5	1420.8							
Response 6	1250.4							

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: JAU6476-S-methyl Day 0 biomass (72-h)
Test Start Date: Test Ending Date:
Test Species: Selenastrum capricornutum
Test Duration: 96 hours
DATA FILE: SelbioS0.icp
OUTPUT FILE: SelbioS0.i05

Conc. ID	Number Replicates	Concentration mg/L	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	1380.200	75.440	1380.200
2	3	1.030	1161.600	27.126	1161.600
3	3	1.600	980.400	52.183	980.400
4	3	2.840	708.400	66.623	708.400
5	3	4.910	468.400	11.085	468.400
6	3	8.740	367.067	62.308	367.067
7	3	15.400	364.800	30.144	364.800
8	3	55.500	280.000	52.818	280.000

The Linear Interpolation Estimate: 0.3252 Entered P Value: 5

Number of Resamplings: 80
The Bootstrap Estimates Mean: 0.3327 Standard Deviation: 0.0465
Original Confidence Limits: Lower: 0.2732 Upper: 0.4394
Expanded Confidence Limits: Lower: 0.2576 Upper: 0.4737
Resampling time in Seconds: 0.05 Random_Seed: 900295420