Data Evaluation Report on the acute toxicity of Penoxsulam on the Freshwater Alga, *Pseudokirchneriella subcapitata*

PMRA Submission #:{.....} EPA MRID #: 45831119

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D288160
	OECD Data Point	{.....}
	EPA MRID	45831119
	EPA Guideline	123-2

Test material:	Penoxsulam	Purity: 98%
Common name:	XDE-638 Metabolite (BSTCA)	
Chemical name:	IUPAC:Not reported	
	CAS name: Not reported	
	CAS No.: Not reported	
	Synonyms: Not reported	

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 12/30/03

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Date: 12/30/03

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{EPA/OECD/PMRA}

Date: {.....} Jgoodyear

Secondary Reviewer(s): {.....}
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Date: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code ~~{99031~~ 119 031

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Hoberg, J.R. 2002. XDE-638 Metabolite (BSTCA): Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Unpublished study performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6173/Dow Study No. 011238. Study submitted by The Dow Chemical Company for Dow AgroSciences, LLC Midland, Michigan. Experimental start date January 3, 2002 and experimental termination date January 7, 2002. The final report issued February 8, 2002.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of ***Pseudokirchneriella subcapitata*** were exposed to Penoxsulam, as XDE-638 Metabolite (BSTCA), under static conditions. The nominal concentrations were 0 (negative and solvent controls), 0.10, 0.26, 0.64, 1.6, 4.0, and 10 mg a.i./L. The mean measured concentrations were <0.026 (LOQ, negative and solvent controls), 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L. The 96-hour cell density percent inhibitions were 6, -14, -9, -6, -1, and -5% for the 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L treatment groups, respectively. The 72-hour growth rate percent inhibitions were 0, 0, -5, -4, -5, -5, and 2% for the 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L treatment groups, respectively. The 72-hour area under the growth curve (biomass) percent inhibitions were 0, -16, -4, -8, -19, and 14% for the 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L treatment groups, respectively. No endpoint was significantly affected by treatment with the metabolite BSTCA, the EC_{50} was >10 mg a.i./L the EC_{05} could not be determined for cell density or biomass, but was >10 mg a.i./L for growth rate, and the NOAEC was 10 mg a.i./L.

The study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata*. This study is classified as Core.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type: Static

Cell Density:
NOAEC: 10 mg a.i./L
EC_{05}: could not determine
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Growth rate:
NOAEC: 10 mg a.i./L
EC_{05}: not determined
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOAEC: 10 mg a.i./L
EC_{05}: could not determine
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD Guideline for Testing of Chemicals #201, Alga, Growth Inhibition Test (OECD, 1984); The EC Guideline Annex V-Method C.3., Algal Inhibition Test (EC, 1997); and U.S. EPA FIFRA Subdivision J Guideline, §123-2 (U.S. EPA, 1982). There were no notable deviations from U.S. EPA Guideline, §123-2.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Penoxsulam, XDE-638 Metabolite (BSTCA)

Description: Not reported

Lot No./Batch No. : E0767-54 and E1145-46

Purity: 98%

Stability of Compound Under Test Conditions: The mean measured concentrations of XDE-638 were 98-106% of nominal at hour 0 and 100-106% of nominal at hour 96 (Table 3, p. 26).

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

Storage conditions of test chemicals: The test substance was stored in a freezer (-20°C).

2. Test organism:

Name: *Pseudokirchneriella subcapitata* (Same as *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. capricornutum, 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: Not reported
Source: Originally from Carolina Biological Supply Company, Burlington, NC. Current in-house laboratory cultures.
Age of inoculum: 3 days old
Method of cultivation: Algal Assay Procedure (AAP) medium (Table 1, p. 24).

B. STUDY DESIGN:

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

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not)	Continuous Algal Assay Procedure (AAP) medium (Table 1, p. 24); same as test.	Inoculum used in test was taken from stock culture and transferred to fresh medium three days before testing.
health: (any toxicity observed)	Not reported	*EPA recommends two week acclimation period.* *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	
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with stainless steel caps 250 ml 100 ml	*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:	Algal Assay Procedure (AAP) medium 7.1-7.5 8.6-8.7 Yes $NaHCO_3$	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.*

Parameter	Details	Remarks *Criteria*
Salinity **(for marine algae):**	N/A	*EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water Sterilized 7.5 ± 0.1 N/A None 0.47 mg a.i./L Not reported Not reported Not detected 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	
Aeration or agitation	Agitation, 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 Selenastrum capricornutum, 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:	 3 3 3	*EPA requires a negative and/or solvent control with 3 or more*

Parameter	Details	Remarks *Criteria*
		replicates per doses. Navicula sp. tests should be conducted with four replicate. *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.*
Test concentrations nominal: measured:	0 (negative and solvent controls), 0.10, 0.26, 0.64, 1.6, 4.0, and 10 mg a.i./L <0.026 (LOQ, negative and solvent controls), 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L	The mean measured concentration was determined for the highest treatment group (Table 3, p. 26). *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:	23-24°C Continuous 3200-5400 lux, fluorescent lighting	*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*

Parameter	Details	Remarks
		Criteria
		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:	N/A	
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 count, area under the growth curve (biomass), and growth rate	Biomass and growth rates were determined for up to 72 hours of exposure. *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	Haemocytometer with a compound microscope	*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).*

Parameters	Details	Remarks/Criteria
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 and solvent control group cell densities at test termination was 159X and 166X greater, respectively than the dilution water and solvent control group 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 6, -14, -9, -6, -1, and -5% for the 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L treatment groups, respectively. The 72-hour growth rate percent inhibitions were 0, 0, -5, -4, -5, -5, and 2% for the 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L treatment groups, respectively. The 72-hour area under the growth curve (biomass) percent inhibitions were 0, -16, -4, -8, -19, and 14% for the 0.10, 0.27, 0.64, 1.7, 4.2, and 10 mg a.i./L treatment groups, respectively.

Table 3: Effect of Penoxsulam, XDE-638 Metabolite (BSTCA), on freshwater alga (*Pseudokirchneriella subcapitata*)

Treatment mean measured and nominal concentrations [a] (mg a.i./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	25,800	1,590,000	--
Solvent control	~10,000	23,300	1,660,000	--
0.10 (0.10)	~10,000	34,200	1,530,000	6
0.27 (0.26)	~10,000	33,300	1,860,000	-14
0.64 (0.64)	~10,000	21,700	1,770,000	-9
1.7 (1.6)	~10,000	22,500	1,730,000	-6
4.2 (4.0)	~10,000	30,800	1,640,000	-1

10 (10)	~10,000	27,500	1,710,000	-5
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.
[b] The % inhibition was based on pooled controls.

Table 4: Effect of Penoxsulam, XDE-638 Metabolite (BSTCA), on the freshwater alga *Pseudokirchneriella subcapitata*

Mean Measured and Nominal Treatment Concentrations [a] (mg a.i./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)[c]
Dilution water control	~10,000	1.34	--	341,000	--
Solvent control	~10,000	1.29	0	299,000	--
0.10 (0.10)	~10,000	1.29	0	320,000	0
0.27 (0.26)	~10,000	1.36	-5	371,000	-16
0.64 (0.64)	~10,000	1.34	-4	334,000	-4
1.7 (1.6)	~10,000	1.35	-5	347,000	-8
4.2 (4.0)	~10,000	1.35	-5	380,000	-19
10 (10)	~10,000	1.26	2	276,000	14
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] The percent inhibitions for growth rates were compared to the solvent control.
[c] The percent inhibitions for biomass were compared to the pooled controls.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density[b]
NOAEC or EC_{05} (mg a.i./L)	10	10	10
EC_{50} (mg a.i./L)	>10	>10	>10
IC_{25}/EC_{25}	Not reported	Not reported	Not reported
Reference chemical, if used			

NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

N/A = Not applicable.
[a] The biomass and growth values based on 72 hour data.
[b] The cell density values based on 96 hour data.

B. REPORTED STATISTICS:

Statistical Method: The formulas used to calculate the area under the growth curve and growth rates are presented on p. 16. A t-test was used to compare the dilution water (negative) and solvent controls. The controls were pooled for cell density and biomass statistical analyses. The growth rate treatments were compared to the solvent control. The data was analyzed for normality using the Shapiro-Wilk's Test and homogeneity of variance using Bartlett's Test. The Williams' test was used to compare the treatment groups to the pooled or solvent control. The cell density values were based on 96 hour data. The biomass and growth values were based on 72 hour data. The reported statistics were based on the mean measured test concentrations.

Cell Density:
NOAEC: 10 mg a.i./L
EC_{05}: Not reported
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Growth rate:
NOAEC: 10 mg a.i./L
EC_{05}: Not reported
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOAEC: 10 mg a.i./L
EC_{05}: Not reported
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density and biomass data satisfied the assumptions of ANOVA. The NOAEC values for these endpoints were determined using this test via TOXSTAT statistical software. The Probit method could not be used to determine the EC_{05} values for cell density or biomass because of the non-linear pattern of these responses. The toxicity values for growth rate could be visually determined, as there was no significant inhibition (>2%) of this endpoint. The reviewer used the mean measured concentrations to calculate toxicity values.

Cell Density:

NOAEC: 10 mg a.i./L
EC_{05}: could not determine
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Growth rate:

NOAEC: 10 mg a.i./L
EC_{05}: not determined
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOAEC: 10 mg a.i./L
EC_{05}: could not determine
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

N/A

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study author; there was no significant effect of the metabolite BSTCA on any algal endpoint.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* [§123-2]. This study is classified as Core. There were no significant effects on cell density, growth rate or biomass. The EC_{50} was >10 mg a.i./L and the NOAEC was 10 mg a.i./L for all endpoints.

Cell Density:
NOAEC: 10 mg a.i./L
EC_{05}: could not determine
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Growth rate:
NOAEC: 10 mg a.i./L
EC_{05}: not determined
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOAEC: 10 mg a.i./L
EC_{05}: could not determine
EC_{50}: >10 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None

III. REFERENCES:

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Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessier, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Kiemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinatti, OH.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 1119cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	2156.667	359.444	1.484
Within (Error)	17	4118.667	242.275	
Total	23	6275.333		

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

cell density
File: 1119cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	162.667	162.667		
2	0.10	153.000	153.000	0.878	
3	0.27	185.667	185.667	-2.090	
4	0.64	177.000	177.000	-1.302	
5	1.7	173.000	173.000	-0.939	
6	4.2	164.333	164.333	-0.151	
7	10	171.000	171.000	-0.757	

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

cell density
File: 1119cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	0.10	3	29.222	18.0	9.667
3	0.27	3	29.222	18.0	-23.000
4	0.64	3	29.222	18.0	-14.333
5	1.7	3	29.222	18.0	-10.333

6	4.2	3	29.222	18.0	-1.667
7	10	3	29.222	18.0	-8.333

cell density
File: 1119cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	162.667	162.667	159.444
2	0.10	3	153.000	153.000	159.444
3	0.27	3	185.667	185.667	174.200
4	0.64	3	177.000	177.000	174.200
5	1.7	3	173.000	173.000	174.200
6	4.2	3	164.333	164.333	174.200
7	10	3	171.000	171.000	174.200

cell density
File: 1119cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	159.444				
0.10	159.444	0.293		1.74	k= 1, v=17
0.27	174.200	1.048		1.82	k= 2, v=17
0.64	174.200	1.048		1.85	k= 3, v=17
1.7	174.200	1.048		1.87	k= 4, v=17
4.2	174.200	1.048		1.87	k= 5, v=17
10	174.200	1.048		1.88	k= 6, v=17

s = 15.565
Note: df used for table values are approximate when v > 20.

EC_x
!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

biomass
File: 1119b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	228.926	38.154	2.696
Within (Error)	17	240.593	14.153	
Total	23	469.520		

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

biomass
File: 1119b Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	32.033	32.033		
2	0.10	32.000	32.000	0.013	
3	0.27	37.067	37.067	-1.892	
4	0.64	33.400	33.400	-0.514	
5	1.7	34.700	34.700	-1.002	
6	4.2	38.033	38.033	-2.255	
7	10	27.567	27.567	1.679	

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

biomass
File: 1119b Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	0.10	3	7.063	22.0	0.033
3	0.27	3	7.063	22.0	-5.033
4	0.64	3	7.063	22.0	-1.367
5	1.7	3	7.063	22.0	-2.667
6	4.2	3	7.063	22.0	-6.000
7	10	3	7.063	22.0	4.467

biomass
File: 1119b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	32.033	32.033	34.181
2	0.10	3	32.000	32.000	34.181
3	0.27	3	37.067	37.067	34.181
4	0.64	3	33.400	33.400	34.181
5	1.7	3	34.700	34.700	34.181
6	4.2	3	38.033	38.033	34.181
7	10	3	27.567	27.567	27.567

biomass
File: 1119b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE P=.05 WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	34.181				
0.10	34.181	0.807		1.74	k= 1, v=17
0.27	34.181	0.807		1.82	k= 2, v=17
0.64	34.181	0.807		1.85	k= 3, v=17
1.7	34.181	0.807		1.87	k= 4, v=17
4.2	34.181	0.807		1.87	k= 5, v=17
10	27.567	1.679		1.88	k= 6, v=17

s = 3.762
Note: df used for table values are approximate when v > 20.

EC_x
!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.