Data Evaluation Report on the acute toxicity of Penoxsulam metabolite (5-Hydroxy-XDE-638) on the Algae, *Pseudokirchneriella subcapitata*

PMRA Submission #:{.....} EPA MRID #: 45831118

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	{.....}
EPA MRID	45831118
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: Penoxsulam metabolite (5-Hydroxy-XDE-638) **Purity:** >99% (used as 100%)
Common name: 5-Hydroxy-XDE-638
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: 12/29/03

QC Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation

Signature: Dana Worcester
Date: 12/29/03

Primary Reviewer: ~~Bill Erickson~~ J GOODYEAR **{EPA/OECD/PMRA}**

Date: {.....} Jgoodyear

Secondary Reviewer(s): {.....} **{EPA/OECD/PMRA}**

Date: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code ~~199031~~ 119 031

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Hoberg, J.R. 2002. 5-Hydroxy-XDE-638: Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Unpublished study performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6165/Dow Study No. 011232. Study submitted by The Dow Chemical Company for Dow AgroSciences, LLC Midland, Michigan. Experimental start date December 27, 2001 and experimental termination date December 31, 2001. The final report issued February 7, 2002.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to Penoxsulam, as 5-Hydroxy-XDE-638, 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 <0.014-0.015 (<LOQ, negative and solvent controls), 0.10, 0.25, 0.62, 1.5, 4.0, and 10.0 mg a.i./L. The 96-hour cell density percent inhibitions were 0, 2, 0, 15, 10, and 18% in the 0.10, 0.25, 0.62, 1.5, 4.0, and 10 mg a.i./L treatment groups, respectively. The area under the growth curve (0 to 72 hours) percent inhibitions were 2, -3, -2, 3, -2, and 0% in the 0.10, 0.25, 0.62, 1.5, 4.0, and 10 mg a.i./L treatment groups, respectively. The growth rate (0 to 72 hours)percent inhibitions were 4, -6, -15, -3, -15, and -8% in the 0.10, 0.25, 0.62, 1.5, 4.0, and 10 mg a.i./L treatment groups, respectively. The EC_{50} was >10 mg a.i./L for all endpoints, the EC_{05} was not determined and the NOAEC was 10.0 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: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: 0.594±0.354

Growth rates:
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: 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 (5-Hydroxy-XDE-638)

Description: Not reported

Lot No./Batch No. : F0512-129A

Purity: >99% (used as 100%)

Stability of Compound Under Test Conditions: The mean measured concentrations of 5-Hydroxy-XDE-638 were 100-110% of nominal at hour 0 and 92-100% 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: Stored at room temperature in the dark.

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:	Continuous	Inoculum used in test was taken from stock culture and transferred to fresh medium three days before testing.
culturing media and conditions: (same as test or not)	Algal Assay Procedure (AAP) medium (Table 1, p. 24).	*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	
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 250 ml 100 ml	Test vessels covered with stainless steel caps. *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):**	Algal Assay Procedure (AAP) medium 7.0-7.6 9.7-10.1 Na_2EDTA $NaHCO_3$ 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.*

Parameter	Details	Remarks *Criteria*
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 pH adjusted with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide, if necessary. 1.0 mg a.i./L (December 2001). Not reported Not detected 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 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:	 3 3 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.*

Parameter	Details	Remarks *Criteria*
		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 <0.014-0.015 (<LOQ, negative and solvent controls), 0.10, 0.25, 0.62, 1.5, 4.0, and 10.0 mg a.i./L.	*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)	DMF, 0.10 ml/L	
Method and interval of analytical verification	HPLC; 0 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	24°C Continuous 3700-4300 lux, fluorescent light.	*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:	N/A	

Parameter	Details	Remarks *Criteria*
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 and growth rates were calculated).	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	Cell counts using a 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).*
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 and solvent control cell densities at test termination were both 91X greater than the dilution water control and solvent control cell	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.*

Parameters	Details	Remarks/Criteria
	densities at test initiation.	*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 0, 2, 0, 15, 10, and 18% in the 0.10, 0.25, 0.62, 1.5, 4.0, and 10 mg a.i./L treatment groups, respectively. The area under the growth curve (0 to 72 hours) percent inhibitions were 2, -3, -2, 3, -2, and 0% in the 0.10, 0.25, 0.62, 1.5, 4.0, and 10 mg a.i./L treatment groups, respectively. The growth rate (0 to 72 hours)percent inhibitions were 4, -6, -15, -3, -15, and -8% in the 0.10, 0.25, 0.62, 1.5, 4.0, and 10 mg a.i./L treatment groups, respectively.

Table 3: Effect of Penoxsulam metabolite (5-Hydroxy-XDE-638) on Algae (*Pseudokirchneriella subcapitata*)

Treatment 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	32,000	910,000	--
Solvent control	~10,000	28,000	910,000	--
0.10 (0.10)	~10,000	28,000	910,000	0
0.25 (0.26)	~10,000	32,000	890,000	2
0.62 (0.64)	~10,000	38,000	910,000	0
1.5 (1.6)	~10,000	36,000	770,000	15
4.0 (4.0)	~10,000	36,000	820,000	10
10.0 (10)	~10,000	34,000	750,000*	18
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibitions were comparisons of the treatment groups to the pooled control.

*Significantly reduced compared to pooled control (Williams' Test).

Table 4: Effect of Penoxsulam metabolite (5-Hydroxy-XDE-638) on Algae *(Pseudokirchneriella subcapitata)*

Treatment day 0 measured and 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)[b]
Dilution water control	~10,000	1.14	--	230,000	--
Solvent control	~10,000	1.15	--	240,000	--
0.10 (0.10)	~10,000	1.12	2	220,000	4
0.25 (0.26)	~10,000	1.17	-3	250,000	-6
0.62 (0.64)	~10,000	1.16	-2	270,000	-15
1.5 (1.6)	~10,000	1.11	3	240,000	-3
4.0 (4.0)	~10,000	1.16	-2	270,000	-15
10.0 (10)	~10,000	1.14	0	250,000	-8
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 comparisons of the treatment groups to the pooled control. Negative percent inhibition indicates promoted growth.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density[b]
NOAEC (mg a.i./L)	10	10	4.0
EC_{05} (mg a.i./L)	ND	ND	ND
EC_{50} (mg a.i./L)	>10	>10	>10
IC_{25}/EC_{25} (mg a.i./L) (95% C.I.)	Not Reported	Not Reported	Not Reported
Reference chemical, if used NOAEC IC_{25}/EC_{25}	N/A	N/A	0.026 mg a.i./L

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

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 all statistical analyses. 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 control. The cell density values based on 96 hour data. The biomass and growth values based on 72 hour data. The reported statistics were based on the mean measured test concentrations.

Cell density:
$NOAEC/EC_{05}$: 4.0 mg a.i./L 95% C.I.: N/A
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A

Growth rates:
$NOAEC/EC_{05}$: 10.0 mg a.i./L 95% C.I.: N/A
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A

Plant biomass (area under the growth curve):
$NOAEC/EC_{05}$: 10.0 mg a.i./L 95% C.I.: N/A
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: Cell density

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density data satisfied the assumptions of ANOVA. The negative and solvent controls were compared using a Student's t-test and, upon finding no difference, the two were pooled for comparison to treatment. The NOAEC was determined using this test via TOXSTAT statistical software. The EC_{05} value for cell density was determined using the Probit method via Nuthatch statistical software; the EC_{50} value could be visually determined because inhibition of cell density did not exceed 50%. Biomass and growth rate data were not analyzed statistically, as the toxicity values could be visually determined because of the minimal inhibition (<5%) exhibited by these endpoints. The reviewer used the mean measured concentrations to calculate toxicity values.

Cell density:
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: 0.594±0.354

Growth rates:
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

N/A

E. REVIEWER'S COMMENTS:

The reviewer's analysis did not detect a significant inhibition for cell density at the highest treatment level; otherwise, the reviewer's results were identical to those of the study author. The reviewer's conclusions are reported in the Executive Summary and Conclusions sections.

The 96 hour concentration of 5-hydroxy-XDE-638 was compared with and without algae using the 1.6 mg a.i./L treatment level.

The test was conducted according to U.S. EPA Good Laboratory Practice Regulations with the following exception: the data for routine water contaminant screening analysis was not collected in accordance to GLP procedures. A GLP statement was provided.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* [Subdivision J, §123-2]. This study is classified as Core. There were no significant effects of 5-Hydroxy-XDE-638 on any endpoint. The EC_{50} was >10 mg a.i./L for all endpoints and the NOAEC was 10 mg a.i./L. The EC_{05} was not determined.

Cell density:
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: 0.594±0.354

Growth rates:
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 10.0 mg a.i./L
EC_{05}: ND
EC_{50}/IC_{50}: >10 mg a.i./L 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None

III. REFERENCES:

ASTM. 2000. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians.

Standard E729-88a, American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, Pennsylvania.

EC, 1997. Official Journal of the European Communities. January 1997. Annex V. Part C: Methods for the Determination of Ecotoxicity. Method C.3. Algal Inhibition Test.

Horning, W.B. and C.I. Weber, 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-89/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Miller, W.E., J.C. Green and T. Shiroyama. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA 600/9-78-018. U.S. Environmental Protection Agency, Corvallis, Oregon.

OECD. 1997. Good Laboratory Practices as acknowledged in the EEC Council Directive 88/320/EEC of 9 June 1988.

OECD. 1984. OECD Guideline for Testing of Chemicals. Alga, Growth Inhibition Test. Guideline #201. Adopted 7 June, 1984.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry.* 2nd Edition. W.H. Freeman and Co. New York. 859 pp.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants. Report No EPA 540/9-82-020, PB83-153940. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test guideline, OPPTS 850.5400. Algal Toxicity, Tiers I and II. "Public Draft" EPA 712-C-96-164. April 1996. U.S. Environmental Protection Agency. Washington, D.C.

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.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27: 103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28: 519-531.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 1118cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	966.125	161.021	2.121
Within (Error)	17	1290.833	75.931	
Total	23	2256.958		

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

cell density
File: 1118cd 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	90.833	90.833		
2	0.10	91.333	91.333	-0.081	
3	0.25	89.333	89.333	0.243	
4	0.62	91.000	91.000	-0.027	
5	1.5	77.000	77.000	2.245	
6	4.0	82.000	82.000	1.434	
7	10.0	75.333	75.333	2.516	

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

cell density
File: 1118cd 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	16.359	18.0	-0.500
3	0.25	3	16.359	18.0	1.500
4	0.62	3	16.359	18.0	-0.167

5	1.5	3	16.359	18.0	13.833
6	4.0	3	16.359	18.0	8.833
7	10.0	3	16.359	18.0	15.500

cell density
File: 1118cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	90.833	90.833	91.000
2	0.10	3	91.333	91.333	91.000
3	0.25	3	89.333	89.333	90.167
4	0.62	3	91.000	91.000	90.167
5	1.5	3	77.000	77.000	79.500
6	4.0	3	82.000	82.000	79.500
7	10.0	3	75.333	75.333	75.333

cell density
File: 1118cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE WILLIAMS P=.05	DEGREES OF FREEDOM
GRPS 1&2 POOLED	91.000				
0.10	91.000	0.027		1.74	k= 1, v=17
0.25	90.167	0.108		1.82	k= 2, v=17
0.62	90.167	0.108		1.85	k= 3, v=17
1.5	79.500	1.839		1.87	k= 4, v=17
4.0	79.500	1.839		1.87	k= 5, v=17
10.0	75.333	2.516	*	1.88	k= 6, v=17

s = 8.714
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err. /Estimate	Lower Bound
EC5	**0.58**	**0.015**	**23.**	**0.77**	**0.026**
EC10	2.4	0.26	21.	0.46	0.11
EC25	25.	3.2	1.9E+02	0.43	0.13
EC50	3.4E+02	2.7	4.3E+04	1.0	0.0079

Slope = 0.594 Std.Err. = 0.354

Goodness of fit: p = 0.56 based on DF= 4.0 17.

1118CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	90.8	91.6	-0.729	100.	0.00
0.100	3.00	91.3	89.9	1.42	98.2	1.80
0.250	3.00	89.3	88.7	0.644	96.9	3.14
0.620	3.00	91.0	86.8	4.19	94.8	5.19
1.50	3.00	77.0	84.2	-7.15	91.9	8.09
4.00	3.00	82.0	80.0	1.96	87.4	12.6
10.0	3.00	75.3	74.9	0.385	81.9	18.1

!!!Warning: EC25 not bracketed by doses evaluated.
!!!Warning: EC50 not bracketed by doses evaluated.