

US EPA ARCHIVE DOCUMENT

Product

Data Evaluation Report on the acute toxicity of Glyphos (~~glyphosate as the IPA salt~~) on the Freshwater Diatom *Navicula pelliculosa*

PMRA Submission #: {.....}

EPA MRID #: 45666701

Data Requirement: PMRA DATA CODE: {.....}
EPA DP Barcode: D283017
OECD Data Point: {.....}
EPA MRID: 45666701
EPA Guideline: 123-2

Document Number



2001004

Test material: *Glyphosate product*
Common name: Glyphos (~~glyphosate as the IPA salt~~)
Chemical name: Glyphos (~~glyphosate as the IPA salt~~)
IUPAC: Not reported
CAS name: Not reported
CAS No.: 1071-83-6
Synonyms: Not reported

Purity: 31.0% *Glyphosate acid*

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: *Rebecca Bryan*
Date: 10/17/02

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation

Signature: *Teri Myers*
Date: 10/17/02

Primary Reviewer: Stephen Carey
{EPA/OECD/PMRA}

Signature: *Stephen Carey*
Date: {.....}
10/29/02

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

Date: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 103601

Date Evaluation Completed: {dd-mm-yyyy}
October 29, 2002

CITATION: Desjardins, D., Sutherland, C., Kendall, T., and Krueger, H. 2001. Glyphos® Herbicide: A 96-Hour Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland; sponsored by Cheminova A/S, Lemvig, Denmark. Wildlife Study No. 232A-101. Experimental initiation date January 4, 2001 and experimental termination date January 15, 2001 (p. 10). Final report issued December 12, 2001.

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom

Navicula pelliculosa

PMRA Submission #: {.....}

EPA MRID #: 45666701

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Navicula pelliculosa* were exposed to Glyphos® herbicide (glyphosate product) under static conditions. Nominal concentrations were 16, 31, 63, 125, 250, and 500 µg glyfos/L. Mean measured concentrations were 20, 36, 71, 136, 270, and 566 µg glyfos/L (equivalent to 6.1, 11, 22, 41, 82, and 172 µg a.e./L of glyphosate acid); response in treatment was compared to response in a dilution water control. Significant reductions in cell density were detected in the 566 µg glyfos/L (172 µg a.e./L) treatment group when compared to the dilution water control. Percent inhibition was -8.3, -16, -18, -9.4, -1.6, and 91% for the 20, 36, 71, 136, 270, and 566 µg glyfos/L treatment groups, respectively. The NOEC was 270 µg glyfos/L (82 µg a.e./L) and the EC₅₀ was 390 µg glyfos/L (120 µg a.e./L), based on cell density.

The study is scientifically sound and it satisfies the guidelines for an aquatic nonvascular plant study with *Navicula pelliculosa* (US EPA FIFRA, Subdivision J, §123-2). This study is classified as CORE for a formulated product.

Results Synopsis

Test Organism: *Navicula pelliculosa*

Test Type: Static

Cell Density:

NOEC: 270 µg glyfos/L (82 µg a.e./L)

EC₅₀: 390 µg glyfos/L (120 µg a.e./L)

Probit: 8.78 ± 1.37 (8.77 ± 1.36)

LOEC: 566 µg glyfos/L (172 µg a.e./L)

95% C.I.: 350-440 µg glyfos/L (110-130 µg a.e./L)

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom

Navicula pelliculosa

PMRA Submission #: {.....}

EPA MRID #: 45666701

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Guideline Subdivision J, §123-2. The following deviations are noted:

1. Test medium was agitated which is not recommended for this species.
2. The study author failed to report the OECD test chemical physical characteristics (i.e., water solubility, vapor pressure, molecular weight and specific activity).

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

A. MATERIALS:

1. Test Material Glyfos

Description: Yellow liquid

Lot No./Batch No. : 80821-47

Purity: 31.0%

Stability of Compound

Under Test Conditions: Mean measured concentrations of Glyphos ranged from 104 to 118% of nominal concentrations on day 0 and ranged from 108 to 133% on Day 4. OECD requirements were not reported.

Water solubility: Not reported

Vapor pressure: Not reported

Specific activity: Not reported

Molecular weight: Not reported

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

Storage conditions of test chemicals: The test chemical was stored in the dark at room temperature.

2. Test organism:

Name: *Navicula pelliculosa*

Strain: Not reported

Source: Laboratory stock cultures

Age of inoculum: ≥ 14 days

Method of cultivation: Freshwater algal medium with silica and selenium constituents.

B. STUDY DESIGN:

a) Range-finding Study: A previous range-finding study was conducted in order to estimate the nominal concentration range for the definitive study. The results were not reported.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	≥ 14 days Freshwater algal medium with silica and selenium constituents; same as test Algal cells were actively growing.	<hr/> 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	Incubator	
Duration of the test	96 hours and 7-day recovery phase	<hr/> EPA requires: 96 - 120 hours OECD: 72 hours
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 mL (Erlenmeyer flask) 100 mL	<hr/> OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom

Navicula pelliculosa

PMRA Submission #:{.....}

EPA MRID #: 45666701

Parameter	Details	Remarks
		Criteria
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Freshwater algal medium with silica and selenium constituents. 7.3 7.6-8.2 0.300 mg/L Na ₂ EDTA•2H ₂ O 15.00 mg/L NaHCO ₃ N/A	<hr/> 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 and no chelators.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes (Appendix 1, p. 33)	
Dilution water source: type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Wildlife International, Ltd. well water NANOpure® water, freshwater algal medium 7.5 N/A Purified well water with reagent-grade chemicals. Not reported Not reported Below levels of concern (Appendix 2, pp. 34-35) Not detected Not reported	<hr/> EPA pH: <i>Skeletonema costatum</i> = ~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 solution	
Aeration or agitation	Agitation (100 oscillations per minute)	Agitation not recommended for this species. <hr/> EPA recommends agitation only for <i>Selenastrum</i> at 100 cycles per min and <i>Skeletonema</i> at ~60 cycles per min. Aeration is not recommended.

US EPA ARCHIVE DOCUMENT

Parameter	Details	Remarks
		Criteria
Initial cells density	10,000 cells/mL	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i> cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
Number of replicates control: solvent control: treated ones:	4 N/A 4	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</p> <p>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.</p>
Test concentrations nominal: measured:	<p>16, 31, 63, 125, 250, and 500 µg glyphos/L.</p> <p>20, 36, 71, 136, 270, and 566 µg glyphos/L (6.1, 11, 22, 41, 82, and 172 µg ae/L).</p>	<p>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</p> <p>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.</p>

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom

Navicula pelliculosa

PMRA Submission #:{.....}

EPA MRID #: 45666701

Parameter	Details	Remarks
		Criteria
Solvent (type, percentage, if used)	None.	
Method and interval of analytical verification	HPLC; 0 and 96 hours	
Test conditions temperature: photoperiod: light intensity and quality:	24 ± 2°C (range: 23.8-25.3°C) continuous light 3920-4240 lux, cool-white flourescent lighting	<i>EPA temperature: <u>Skeletonema</u>: 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%)</i> <i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i>
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 (cell density)	<i>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.</i>

Parameters	Details	Remarks/Criteria
Measurement technique for cell density and other end points	Hemacytometer and microscope	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>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).</i>
Observation intervals	Every 24 hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	Recovery phase for 7 days (nominal 500µg glyphos/L treatment group)	
Indicate whether there was exponential growth in the control	Yes, dilution water control group cell density at test termination was 121.8X greater than the control group cell density at test initiation.	<i>EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The decrease in the mean cell count was significant ($\alpha = 0.05$) at the 566 µg/L treatment group when compared to the dilution water control. The percent inhibitions were -8.3, -16, -18, -9.4, -1.6, and 91% for the 20, 36, 71, 136, 270, and 566 µg/L treatment groups, respectively.

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom *Navicula pelliculosa*

PMRA Submission #:{.....}

EPA MRID #: 45666701

Table 3: Effect of Glyphos (glyphosate product) on diatom growth (*Navicula pelliculosa*)

Measured and nominal concentrations ^a (µg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					cell count	% inhibition ^b
Dilution water control	~10,000	57,836	157,704	1,155,694	1,217,809	--
Solvent control	N/A	N/A	N/A	N/A	N/A	N/A
20 (16)	~10,000	57,407	258,432	1,205,577	1,319,364	-8.3
36 (31)	~10,000	63,119	211,092	998,386	1,410,708	-16
71 (63)	~10,000	51,828	250,534	1,330,550	1,431,142	-18
136 (125)	~10,000	42,886	106,723	647,069	1,331,769	-9.4
270 (250)	~10,000	30,249	91,065	319,765	1,237,214	-1.6
566 (500)	~10,000	10,682	15,236	34,752	107,645	91*
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A	N/A

^a Mean measured concentrations of Glyphos formulation. Nominal concentrations are in parentheses.

^b % inhibition was determined by comparing the treatment groups to the dilution water control.

* Significantly different ($\alpha = 0.05$) from dilution water control.

Table 4: Effect of Glyphos (glyphosate product) on the diatom, *Navicula pelliculosa*

Measured and nominal concentrations ^a (µg/L)	Initial cell density (cells/mL)	Mean Growth Rate per day ^b	% inhibition (Mean Growth Rate per day)	Mean Area Under Growth Curve ^b	% inhibition (Mean Area Under Growth Curve)
Dilution water control	~10,000	NR	NR	NR	NR
Solvent control	~10,000	N/A	N/A	N/A	N/A
0.0089 (0.010)	~10,000	NR	NR	NR	NR
0.018 (0.020)	~10,000	NR	NR	NR	NR
0.043 (0.040)	~10,000	NR	NR	NR	NR
0.093 (0.080)	~10,000	NR	NR	NR	NR
0.17 (0.16)	~10,000	NR	NR	NR	NR

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom *Navicula pelliculosa*

PMRA Submission #: {.....}

EPA MRID #: 45666701

Measured and nominal concentrations ^a (µg/L)	Initial cell density (cells/mL)	Mean Growth Rate per day ^b	% inhibition (Mean Growth Rate per day)	Mean Area Under Growth Curve ^b	% inhibition (Mean Area Under Growth Curve)
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

^a Mean measured concentrations of Glyphos formulation. Nominal concentrations are in parentheses.

NR Not Reported

Table 5: Statistical endpoint values.

Statistical Endpoint	Cell density	Growth rate	Area under growth curve (Biomass)
NOEC or EC ₀₅ (µg/L)	270	N/A	N/A
EC ₅₀ (µg/L) (95% C.I.)	392 (346-443 µg/L)	N/A	N/A
other (IC ₂₅ /EC ₂₅)	N/A	N/A	N/A
Reference chemical, if used NOEC IC ₅₀ /EC ₅₀	N/A	N/A	N/A

N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: Data was evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Levene's test. ANOVA and Dunnett's test was used to compare treatment groups to dilution water control. The EC₅₀ was determined using the non-linear regression or linear interpolation via SAS software; the EC₅₀ and 95% confidence interval for the glyphosate acid equivalent of the IPA salt concentrations were converted from these values using the equation provided on p. 38.

Cell Density:

NOEC: 270 µg/L (82 µg a.i./L)
 EC₅₀: 392 µg/L (119 µg a.i./L) 95% C.I.: 346-443 µg/L (105-135 µg a.i./L)
 Endpoint(s) Affected: Cell density

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOEC and LOEC for cell density were determined using ANOVA, followed by Dunnett's test via TOXSTAT statistical software after confirming that the data were normally distributed and the variances were homogeneous. The EC₅₀ values for the formulated end-use product and the glyphosate acid equivalent concentrations were estimated by conducting two separate analyses to compare both sets of these concentrations to the cell density response using the probit method via Nuthatch statistical software. In most cases this method of

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom *Navicula pelliculosa*

PMRA Submission #: {.....}

EPA MRID #: 45666701

analysis provided slightly different values than simply using the glyphosate equivalent equation on p. 38 to convert the formulated end-use product estimates, because the slope of the dose-response relationship slightly changed..

Cell Density:

NOEC: 270 µg glyfos/L (82 µg a.e./L)

LOEC: 566 µg glyfos/L (172 µg a.e./L)

EC₅₀: 390 µg glyfos/L (120 µg a.e./L)

95% C.I.: 350-440 µg glyfos/L (110-130 µg a.e./L)

Probit: 8.78 ± 1.37 (8.77 ± 1.36)

D. STUDY DEFICIENCIES:

The test medium was agitated (100 rpm). This and other minor deviations (mentioned above) did not affect the acceptability or the validity of this study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the EC₅₀ for cell density based on both the formulated product and the glyphosate acid (active ingredient) concentrations differed slightly from the study authors' (390 vs. 392 µg a.e./L and 120 vs. 119 µg a.e./L). These differences were due to the fact that the reviewer estimated the EC₅₀ value and 95% confidence interval for the formulated end-use product concentrations and glyphosate acid concentrations separately, while the study authors simply used the equation given in the study to convert the formulated product EC₅₀ to reflect acid equivalent (active ingredient) concentrations. These methods produced a slightly different set of numbers because the slope of the dose-response relationship marginally changed upon re-analysis of the values with the converted acid equivalent concentrations.

The dilution water and nominal 500 µg glyfos/L treatment group were used in the recovery phase. Samples were collected at the end of the 96-hour test. After 7 days, the algal growth had recovered from test chemical effects and recovery phase was terminated.

The measured concentrations 20, 36, 71, 136, 270, and 566 µg glyfos/L were equivalent to 6.1, 11, 22, 41, 82, and 172 µg a.e./L of the active ingredient.

The derivatization method for glyphosate (isopropylamine salt) analysis used in this study was evaluated in MRID 45666705. Verification samples were fortified and analyzed in freshwater algal medium. The concentrations verified ranged from 15.0 to µg glyfos/L to 15,000 µg glyfos/L (equivalent to 4.56 to 4,560 µg a.e./L of glyphosate (isopropylamine salt). The mean recoveries were 103-108% (mean recovery of 105%) which indicates the analytical method is valid for quantification of glyphosate (isopropylamine salt).

F. CONCLUSIONS: The study is scientifically sound and it satisfies the guidelines for an aquatic nonvascular plant study with *Navicula pelliculosa* (US EPA FIFRA, Subdivision J, §123-2). This study is classified as CORE for a formulated product. Significant reductions in cell density were detected at the highest treatment level, 566 µg glyfos/L (172 µg acid/L).

Cell Density:

NOEC: 270 µg glyfos/L (82 µg a.e./L)

LOEC: 566 µg glyfos/L (172 µg a.e./L)

EC₅₀: 390 µg glyfos/L (120 µg a.e./L)

95% C.I.: 350-440 µg glyfos/L (110-130 µg a.e./L)

Probit: 8.78 ± 1.37 (8.77 ± 1.36)

Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom

Navicula pelliculosa

PMRA Submission #: {.....}

EPA MRID #: 45666701

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400: Algal Toxicity Tiers I and II.

ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-hour Toxicity Tests with Microalgae*. American Society for Testing and Materials. Philadelphia, Pennsylvania.

The SAS System for Windows. 1999. Version Eight. SAS Institute, Inc. Cary, North Carolina.

Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry*. 11: 1485-1494.

Norberg-King, T.J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach*. Version 2.0 U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, Minnesota. Technical Report 03-93.

Cohen, Jacob. 1977. *Statistical Power Analysis for the Behavioral Sciences*. Academic Press, New York.

Kendall, T. and Nixon, W. 2001. Analytical Method Verification for the Determination of Glyphos® [glyphosate as the isopropylamine (IPA) salt] Herbicide in Freshwater Algal Medium. Wildlife International Ltd. Study No.: 232C-101. MRID No. 45666705.

US EPA ARCHIVE DOCUMENT

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
 File: 6701cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3922311.768	653718.628	158.681
Within (Error)	14	57675.974	4119.712	
Total	20	3979987.742		

Critical F value = 2.85 (0.05,6,14)
 Since F > Critical F REJECT Ho:All groups equal

cell density
 File: 6701cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1217.809	1217.809		
2	20 ug/L	1319.364	1319.364	-1.938	
3	36 ug/L	1410.708	1410.708	-3.681	
4	71 ug/L	1431.142	1431.142	-4.071	
5	136 ug/L	1331.769	1331.769	-2.175	
6	270 ug/L	1237.214	1237.214	-0.370	
7	566 ug/L	107.645	107.645	21.184	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

cell density
 File: 6701cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	20 ug/L	3	132.589	10.9	-101.555
3	36 ug/L	3	132.589	10.9	-192.899
4	71 ug/L	3	132.589	10.9	-213.332
5	136 ug/L	3	132.589	10.9	-113.959
6	270 ug/L	3	132.589	10.9	-19.405
7	566 ug/L	3	132.589	10.9	1110.164

cell density
 File: 6701cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
-------	----------------	---	---------------	------------------	-----------------

**Data Evaluation Report on the acute toxicity of Glyphos (glyphosate product) on the Freshwater Diatom
*Navicula pelliculosa***

PMRA Submission #:{.....}

EPA MRID #: 45666701

1	neg control	3	1217.809	1217.809	1344.756
2	20 ug/L	3	1319.364	1319.364	1344.756
3	36 ug/L	3	1410.708	1410.708	1344.756
4	71 ug/L	3	1431.142	1431.142	1344.756
5	136 ug/L	3	1331.769	1331.769	1331.769
6	270 ug/L	3	1237.214	1237.214	1237.214
7	566 ug/L	3	107.645	107.645	107.645

cell density

File: 6701cd

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
neg control	1344.756				
20 ug/L	1344.756	2.422	*	1.76	k= 1, v=14
36 ug/L	1344.756	2.422	*	1.85	k= 2, v=14
71 ug/L	1344.756	2.422	*	1.88	k= 3, v=14
136 ug/L	1331.769	2.175	*	1.89	k= 4, v=14
270 ug/L	1237.214	0.370		1.90	k= 5, v=14
566 ug/L	107.645	21.184	*	1.91	k= 6, v=14

s = 64.185

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