Data Evaluation Report on the acute toxicity of Glyfos (glyphosate ~~as the isopropylamine salt~~ product) to aquatic vascular plants *Lemna gibba*

PMRA Submission #:{.....} EPA MRID#:45666704

Data Requirement:

PMRA Data Code:	{.....}
EPA DP Barcode:	D283017
OECD Data Point:	{.....}
EPA MRID:	45666704
EPA Guideline:	123-2

Document Number

2001007

Test material: Glyfos (glyphosate ~~as the isopropylamine salt~~ product) Purity: 31.0% glyphosate acid
Common name: Glyfos (glyphosate as the isopropylamine salt)
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 1071-83-6
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
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Signature: Rebecca Bryan
Date: 10/17/02

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Date: {.....} 11/08/02

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

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

Date Evaluation Completed: {dd ~~mmm yyyy~~} November 14th, 2002

CITATION: Desjardins, D., Sutherland, C., Kendall, T., and Krueger, H. 2001. Glyfos® Herbicide: A 7-Day Toxicity Test with Duckweed (*Lemna gibba* G3). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland; sponsored by Cheminova A/S, Lemvig, Denmark. Wildlife Study No. 232A-104. Experimental start date December 13, 2000, and experimental termination date December 20, 2000. Final report issued December 19, 2001.

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Glyfos (glyphosate product) at mean measured concentrations of 0.94, 1.9, 3.9, 7.9, 16, and 30 mg glyfos/L under static conditions. Nominal concentrations were 0.94, 1.9, 3.8, 7.5, 15, and 30 mg glyfos/L. Plant number was significantly reduced at the 16 mg glyfos/L (4.9 mg a.e./L) treatment group; the NOEC for plant number was 7.9 mg glyfos/L (2.4 mg a.e./L) and the EC_{50} was 25 mg glyfos/L (7.7 mg a.e./L). Frond number was affected at lower concentrations than plant number, with significant reductions occurring at the 1.9 mg glyfos/L (0.58 mg a.e./L) treatment group; the NOEC for frond number was 0.94 mg glyfos/L (0.29 mg a.e./L) and the EC_{50} was 27 mg glyfos/L (8.3 mg a.e./L). Percent inhibition for frond number was 4.7, 9.7, 5.3, 4.1, 29, and 56% in the 0.94, 3.9, 7.9, 16, and 30 mg glyfos/L treatment groups, respectively, compared to the dilution water control. At test termination, the percentage of necrotic and chlorotic fronds was higher in the 16 and 30 mg glyfos/L (2.4 and 4.9 mg a.e./L) treatment groups, compared to the dilution water control.

This toxicity study is scientifically sound and satisfy the guideline requirements for an acute toxicity study with aquatic vascular plants. However, the percent recoveries of the test concentrations were 102-113% at test termination. As a result, this study is classified as CORE for a formulated product.

Results Synopsis

Test Organism: *Lemna gibba* G3
Test Type: Static

Plant number
EC_{05}: Not reported 95% C.I.: N/A
NOEC: 7.9 mg glyfos/L (2.4 mg a.e./L) LOEC: 16 mg glyfos/L (4.9 mg a.i/L)
Probit Slope: 4.69 ± 0.660 (4.76 ± 0.669)
EC_{50}/IC_{50}: 25 mg glyfos/L (7.7 mg a.e./L) 95% C.I.: 23-27 mg glyfos/L (7.1-8.3 mg a.e./L)

Frond number
EC_{05}: Not reported 95% C.I.: N/A
NOEC: 0.94 mg glyfos/L (0.29 mg a.e./L) LOEC: 1.9 mg glyfos/L (0.58 mg a.i/L)
Probit Slope: 3.05 ± 0.376 (3.08 ± 0.380)
EC_{50}/IC_{50}: 27 mg glyfos/L (8.3 mg a.e./L) 95% C.I.: 25-30 mg glyfos/L (7.6-9.1 mg a.e./L)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Subdivision J, § 123-2. The following deviations were observed:

1. This study was conducted under static conditions. US EPA recommends that 7-day duckweed tests be conducted under static renewal conditions (1 renewal for a 7-day test).

2. The carbon source of the media was not reported.

3. 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 (glyphosate product)

Description: Yellow liquid

Lot No./Batch No. : 80821-47

Purity: 31.0% glyphosate acid

Stability of Compound
Under Test Conditions: Measured concentrations on day 0 ranged from 95.7 to 104% of nominal concentrations and measured concentrations on day 7 ranged from 102 to 113% of nominal concentrations, showing that the test material was stable under test conditions. 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 material was stored at ambient room temperature.

2. Test organism:

Name: Duckweed, *Lemna gibba* *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: Cultures maintained at Wildlife International, Ltd., Easton, Maryland
Age of inoculum: ≥14 days
Method of cultivation: M-Hoagland's Medium

B. STUDY DESIGN:

a) Range-finding Study: Not reported; the authors indicate that the definitive study was based on the results of an exploratory range-finding test. No further information is provided.

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 Same as test. Prior to the test the plants were actively growing and healthy.	
Test system static/static renewal/ renewal rate for static renewal:	Static	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Environmental chamber	
Duration of the test	7 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel material: (glass/polystyrene) size: fill volume:	 Glass 250 mL 100 mL	
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	 M-Hoagland's medium without EDTA or sucrose 4.6 5.3-5.6 None Not reported	*EPA recommend the following culture media:* *Modified hoagland's E+ or 20X-AAP. Chelators are not recommended.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Wildlife International, Ltd. purified well water 5.0 ± 0.1 Not reported Not reported Not reported Below levels of concern (Appendix 2, pp. 28-29) Not detected Not reported	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported	
Sediment used **(for rooted aquatic vascular plants)** origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	 3 N/A 3	
Number of plants/replicate	5 plants per replicate	*EPA requires 5 plants.*
Number of fronds/plant	3 fronds per plant	*EPA requires 3 fronds per plant.*
Test concentrations nominal: measured:	 0.94, 1.9, 3.8, 7.5, 15, and 30 mg glyfos/L 0.94, 1.9, 3.9, 7.9, 16, and 30 mg glyfos/L (0.29, 0.58, 1.2, 2.4, 4.9, and 9.1 mg a.e./L).	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	None	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification	HPLC; 0 and 7	
Test conditions temperature: photoperiod: light intensity and quality:	24.3-25.2°C Continuous 4750 ± 464 lux (range: 4360-5500 lux), warm-white fluorescent lighting.	*EPA temperature: 25°C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds and toxicity symptoms	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	0, 3, 5, and 7 days.	
Other observations, if any	Colony break-up and root destruction	
Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Mean and replicate data provided	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Mean frond number decreased as test concentrations increased, when compared to the dilution water control. Percent inhibition was 4.7, 9.7, 5.3, 4.1, 29, and 56% in the 0.94, 3.9, 7.9, 16, and 30 mg glyfos/L treatment groups, respectively,

compared to the dilution water control. The mean frond numbers of the 16 and 30 mg glyfos/L treatment groups were significantly reduced when compared to the dilution water control. The significant reduction of the 1.9 mg glyfos/L treatment group was not considered treatment-related.

At test termination, the percentage of necrotic and chlorotic fronds were higher in the 16 and 30 mg glyfos/L treatment groups, compared to the dilution water control.

Table 3: Effect of Glyfos (glyphosate product) on plant and frond number of Duckweed, *Lemna gibba*

Treatment[1] (measured and nominal concentration (mg/L)	Initial plant number/ test solution	Observations		Initial frond number/test solution	Observations	
		Mean plant number	% inhibition		Mean frond number	% inhibition[a]
Dilution water control	15	21	---	45	113.0	---
Solvent control	N/A	N/A	N/A	N/A	N/A	N/A
0.94 (0.94)	15	23	-9.5	45	107.7	4.7
1.9 (1.9)	15	20	4.8	45	102.0*	9.7
3.9 (3.8)	15	23	-9.5	45	107.0	5.3
7.9 (7.5)	15	22	-4.8	45	108.3	4.1
16 (15)	15	19	9.5	45	79.7*	29
30 (30)	15	8	62	45	49.7*	56
Reference chemical (if used)	Not applicable.					

[1] Mean measured concentrations with nominal concentrations reported within parentheses.
[a] % inhibition was determined by comparing the treatment groups to the dilution water control.
* Significantly different ($p<0.05$) from the dilution water control.

Table 4: Statistical endpoint values.

Statistical Endpoint	frond No.	growth rate	other parameter
NOAEC or EC_{05} (mg/L)	7.9	Not applicable	Not applicable
LOAEC (mg/L)	Not reported		
IC_{50} or EC_{50} (mg/L) (95% C.I.)	27 (21 to 33)		
other (IC_{25}/EC_{25})	Not reported		
Reference chemical NOAEC IC_{50}/EC_{50}	Not applicable		

B. REPORTED STATISTICS: Frond number on day 7 was analyzed using mean measured concentrations. The Shapiro-Wilk's test and Bartlett's test were used to confirm the normality and homogeneity of variances. ANOVA and Dunnett's test was used to compare treatment groups to dilution water control. The TOXSTAT statistical program to analyze the plant numbers, frond numbers, and the percentages of dead and necrotic fronds. The EC_{50} and 95% confidence limits were determined through linear interpolation via SAS software; the EC_{50} and 95% confidence interval for the acid equivalent concentrations were converted from these values using the equation provided in the study.

Frond number
NOEC: 7.9 mg glyfos/L (2.4 mg a.i./L) LOEC: Not reported
EC_{50}/IC_{50}: 27 mg glyfos/L (8.2 mg a.i./L) 95% C.I.: 21 - 33 mg glyfos/L (6.4 - 10 mg a.i./L)

C. VERIFICATION OF STATISTICAL RESULTS:

The NOEC and LOEC for plant number and frond number were determined using ANOVA, followed by Dunnett's test via TOXSTAT statistical software. Data were determined to be normally distributed and the variances were homogeneous prior to these analyses. The EC_{50} values for the formulated end-use product and the acid equivalent concentrations were estimated by conducting two separate analyses to compare both sets of these concentrations to the plant number and frond number responses using the probit method via Nuthatch statistical software. In most cases this method of analysis provided slightly different values than simply using the acid equivalent equation in the study to convert the formulated end-use product estimates, because the slope of the dose-response relationship slightly changed.

Plant number
NOEC: 7.9 mg glyfos/L (2.4 mg a.e./L) LOEC: 16 mg glyfos/L (4.9 mg a.i/L)
Probit Slope: 4.69 ± 0.660 (4.76 ± 0.669)
EC_{50}/IC_{50}: 25 mg glyfos/L (7.7 mg a.e./L) 95% C.I.: 23-27 mg glyfos/L (7.1-8.3 mg a.e./L)

Frond number
NOEC: 0.94 mg glyfos/L (0.29 mg a.e./L) LOEC: 1.9 mg glyfos/L (0.58 mg a.i/L)
Probit Slope: 3.05 ± 0.376 (3.08 ± 0.380)
EC_{50}/IC_{50}: 27 mg glyfos/L (8.3 mg a.e./L) 95% C.I.: 25-30 mg glyfos/L (7.6-9.1 mg a.e./L)

D. STUDY DEFICIENCIES:

This study was conducted under static conditions. US EPA recommends that 7-day duckweed tests be conducted under static renewal conditions (1 renewals for a 7-day test). However, the percent recoveries of the test concentrations were 102-113% at test termination.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the EC_{50} for frond number based on the formulated product was identical to the study authors', while the EC_{50} estimate based on the acid equivalent concentrations was slightly different (8.3 vs. 8.2 mg a.e./L). These differences were due to the fact that the reviewer estimated the EC_{50} value and 95% confidence interval for the formulated end-use product concentrations and acid equivalent concentrations separately, while the study authors simply used the equation in the study to convert the formulated product EC_{50} to reflect acid equivalent 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. In this study, the reviewer also determined lower LOEC and NOEC values for this endpoint. The study authors dismissed the significant reduction in frond number at the 1.9 mg glyfos/L (0.58 mg a.e./L) treatment level, because the response for this endpoint was not dose-dependent.

The measured concentrations 0.94, 1.9, 3.9, 7.9, 16, and 30 mg glyfos/L were equivalent to 0.29, 0.58, 1.2, 2.4, 4.9, and 9.1 mg a.e./L of glyphosate acid.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfy the guideline requirements for an acute toxicity study with aquatic vascular plants. However, the percent recoveries of the test concentrations were 102-113% at test termination. As a result, this study is classified as CORE for a formulated product. Frond number was affected at lower concentrations than plant number, with significant reductions occurring at the 1.9 mg glyfos/L (0.58 mg a.e./L) treatment group; the NOEC for frond number was 0.94 mg glyfos/L (0.29 mg a.e./L) and the EC_{50} was 27 mg glyfos/L (8.3 mg a.e./L).

Plant number

NOEC: 7.9 mg glyfos/L (2.4 mg a.e./L)	LOEC: 16 mg glyfos/L (4.9 mg a.i/L)
Probit Slope: 4.69 ± 0.660 (4.76 ± 0.669)	
EC_{50}/IC_{50}: 25 mg glyfos/L (7.7 mg a.e./L)	95% C.I.: 23-27 mg glyfos/L (7.1-8.3 mg a.e./L)

Frond number

NOEC: 0.94 mg glyfos/L (0.29 mg a.e./L)	LOEC: 1.9 mg glyfos/L (0.58 mg a.i/L)
Probit Slope: 3.05 ± 0.376 (3.08 ± 0.380)	
EC_{50}/IC_{50}: 27 mg glyfos/L (8.3 mg a.e./L)	95% C.I.: 25-30 mg glyfos/L (7.6-9.1 mg a.e./L)

Endpoint(s) Affected: frond number

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.4400: *Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II.* Washington, DC.

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..

Microsoft Corporation. Microsoft Excel 2000. Copyright 1985-1999.

West, Inc. and Gulley, D.D. 1996. TOXSTAT Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

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.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

plant number
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	512.286	85.381	44.819
Within (Error)	14	26.667	1.905	
Total	20	538.952		

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

plant number
File: 6704pn 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	21.333	21.333		
2	0.94 mg/L	22.667	22.667	-1.183	
3	1.9 mg/L	20.000	20.000	1.183	
4	3.9 mg/L	23.333	23.333	-1.775	
5	**7.9 mg/L**	**22.333**	**22.333**	**-0.887**	
6	16 mg/L	18.000	18.000	2.958	*
7	30 mg/L	8.000	8.000	11.831	*

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

plant number
File: 6704pn 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	0.94 mg/L	3	2.851	13.4	-1.333
3	1.9 mg/L	3	2.851	13.4	1.333
4	3.9 mg/L	3	2.851	13.4	-2.000
5	7.9 mg/L	3	2.851	13.4	-1.000
6	16 mg/L	3	2.851	13.4	3.333
7	30 mg/L	3	2.851	13.4	13.333

plant number
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	21.333	21.333	22.000
2	0.94 mg/L	3	22.667	22.667	22.000
3	1.9 mg/L	3	20.000	20.000	21.889
4	3.9 mg/L	3	23.333	23.333	21.889
5	7.9 mg/L	3	22.333	22.333	21.889
6	16 mg/L	3	18.000	18.000	18.000
7	30 mg/L	3	8.000	8.000	8.000

plant number
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	22.000				
0.94 mg/L	22.000	0.592		1.76	k= 1, v=14
1.9 mg/L	21.889	0.493		1.85	k= 2, v=14
3.9 mg/L	21.889	0.493		1.88	k= 3, v=14
7.9 mg/L	21.889	0.493		1.89	k= 4, v=14
16 mg/L	18.000	2.958	*	1.90	k= 5, v=14
30 mg/L	8.000	11.832	*	1.91	k= 6, v=14

s = 1.380
Note: df used for table values are approximate when v > 20.

frond number
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	9434.000	1572.333	73.213
Within (Error)	14	300.667	21.476	
Total	20	9734.667		

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

frond number
File: 6704fn 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	113.000	113.000		
2	**0.94 mg/L**	**107.667**	**107.667**	**1.410**	

3	1.9 mg/L	102.000	102.000	2.907	*
4	3.9 mg/L	107.000	107.000	1.586	
5	7.9 mg/L	108.333	108.333	1.233	
6	16 mg/L	79.667	79.667	8.809	*
7	30 mg/L	49.667	49.667	16.738	*

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

frond number
File: 6704fn 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	0.94 mg/L	3	9.573	8.5	5.333
3	1.9 mg/L	3	9.573	8.5	11.000
4	3.9 mg/L	3	9.573	8.5	6.000
5	7.9 mg/L	3	9.573	8.5	4.667
6	16 mg/L	3	9.573	8.5	33.333
7	30 mg/L	3	9.573	8.5	63.333

frond number
File: 6704fn Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	113.000	113.000	113.000
2	0.94 mg/L	3	107.667	107.667	107.667
3	1.9 mg/L	3	102.000	102.000	105.778
4	3.9 mg/L	3	107.000	107.000	105.778
5	7.9 mg/L	3	108.333	108.333	105.778
6	16 mg/L	3	79.667	79.667	79.667
7	30 mg/L	3	49.667	49.667	49.667

frond number
File: 6704fn 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	113.000				
0.94 mg/L	107.667	1.410		1.76	k= 1, v=14
1.9 mg/L	105.778	1.909	*	1.85	k= 2, v=14
3.9 mg/L	105.778	1.909	*	1.88	k= 3, v=14
7.9 mg/L	105.778	1.909	*	1.89	k= 4, v=14
16 mg/L	79.667	8.809	*	1.90	k= 5, v=14
30 mg/L	49.667	16.738	*	1.91	k= 6, v=14

s = 4.634
Note: df used for table values are approximate when v > 20.