FINAL REPORT

Study Title

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST OF TELOMER B 8-2 ALCOHOL (OECD 209)

Test Guideline

OECD (1984) Guideline for Testing of Chemicals, Section 2, No. 209: "Activated Sludge, Respiration Inhibition Test", adopted April 4, 1984

Author

William R. Berti, Ph.D.

Study Completion Date

July 31, 2002

Test Facility

E. I. du Pont de Nemours and Company
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Submitter

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Wilmington, Delaware 19898
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EMSE Study / Project Number

T0105 / 4842

Report Number

EMSER 18-02

PAGE RESERVED FOR SPECIFIC COUNTRY REQUIREMENTS

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the following GLP principles with the following exception as noted below:

- United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160 (effective October 16, 1989)

which are consistent with:

- The OECD Principles on Good Laboratory Practice (ENV/MC/CHEM(98)17) (Paris, 1998)
- Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, (Tokyo, 1984).

The test substance was characterized by the sponsor prior to the initiation of this study. A certificate of analysis was provided. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

The reference substance is a commercially available material. The certificate of analysis (COA) was provided by the supplier and the accuracy of the data is considered sufficient for the purposes of this study.

The stock test solutions were analyzed; however, the analysis was not performed in compliance with GLP. Analysis to confirm concentration and uniformity of the test solutions was not performed. This is believed not to affect the validity of the study because the test and reference substances were accurately measured and added to the test vessels.

Study Director

William R. Berti 31 July-02

William R. Berti, Ph.D.
Date
Senior Research Biologist
DuPont Environmental and Microbiological Sciences & Engineering

Sponsor/Submitter

_____ _____

Date

QUALITY ASSURANCE STATEMENT

Study Number
T0105 / 4842

Study Title
Activated Sludge Respiration Inhibition Test of Glycolic Acid Technical, 70% Solution (OECD 209)

The conduct of this study was inspected periodically.

Audit dates are given below:

Study Phase Inspected	Inspection/Audit Dates	Dates Findings Reported to Study Director	Dates Findings Reported to Management
Study Protocol	11-Feb-2002	16-Feb-2002	16-Feb-2002
Study Conduct	12-Feb-2002	16-Feb-2002	16-Feb-2002
Report	1-July-2002	23-July-2002	26-July-2002

These inspections confirm that the methods, procedures, and observations are accurately and completely described in this report. The reported results accurately and completely reflect the raw data of this study.

Kimberly B. Brebner
Kimberly B. Brebner
QA Auditor
DuPont Co.

31-JUL-2002
Date

CERTIFICATION OF AUTHENTICITY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST OF TELOMER B 8-2 ALCOHOL (OECD 209)

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by:

William R. Berti, Ph.D.
Senior Research Biologist

31 July 2002
Date

Approved by:

John T. Gannon, Ph.D.
Research Manager

07/31/02
Date

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Appendix

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST OF TELOMER B 8-2 ALCOHOL (OECD 209)

William R. Berti, Ph.D.

1.0 SUMMARY

Test System

Telomer B 8-2 Alcohol was tested for toxicity towards activated sludge according to OECD Guideline 209 in the version dated 4-April-1984. For the determination of the toxic behavior of the test substance, activated sludge from the aeration tank of a municipal sewage treatment plant was exposed to the test substance at 100, 320, 1000, 3200, and 10000 µg L^{-1} nominal concentration. After 30 minutes of incubation period the inhibition of the respiration rate of the activated sludge was determined in comparison to a test solution without any test substance.

Findings

Under the conditions of the test, the highest test concentration with 10000 µg test substance in 1 liter tapwater exhibited less than 20% inhibition of the respiration rate, which is the detection limit of the system.

Conclusions

The EC_{50} of the test substance is estimated to be greater than 10000 µg L^{-1} (10 mg L^{-1}).

2.0 GENERAL STUDY INFORMATION

Study Objectives

The aim of this study was the determination of the acute toxic behaviour of Telomer B 8-2 Alcohol towards the microorganisms of activated sludge according to OECD guideline 209 in the version of April 4, 1984. The objectives of this study were to determine the:

- Effect of the test substance, Telomer B 8-2 Alcohol, on microorganisms from municipal sewage sludge and
- Suitable non-inhibitory concentrations of the test substance to be used in biodegradability tests

using a microbial inoculum and an artificial sewage feed and measuring the respiration rate of the test system over a 30 minute time period under controlled laboratory conditions. The purpose of the study was to assess the potential of the test substance to adversely affect aerobic municipal sewage treatment plants.

Test System Justification

The test system is outlined by the OECD guideline 209 and was requested by the submitter.

Study Personnel

E. I. du Pont de Nemours and Company
Environmental and Microbiological Sciences & Engineering and the
Corporate Center for Analytical Sciences

Management:	John T. Gannon, Ph.D.
Study Director:	William R. Berti, Ph.D. E. I. du Pont de Nemours and Company Environmental and Microbiological Sciences & Engineering Central Research & Development, Glasgow Building 300 P.O. Box 6101 Newark, DE 19714-6101 USA
Analytical Chemist:	Daryl P. Cobranchi, Ph.D. E. I. du Pont de Nemours and Company Corporate Center for Analytical Research Central Research & Development, Glasgow Building 300 P.O. Box 6101 Newark, DE 19714-6101 USA
Technical Personnel:	Mark Starr Patrick W. Folsom

Study Execution Dates

Study Initiation Date:	11-Feb-2002
Experimental Start Date:	11-Feb-2002
Experimental Completion Date:	25-Mar-2002
Study Completion Date:	31-July-2002

3.0 MATERIALS AND METHODS

3.1 Test Guidelines

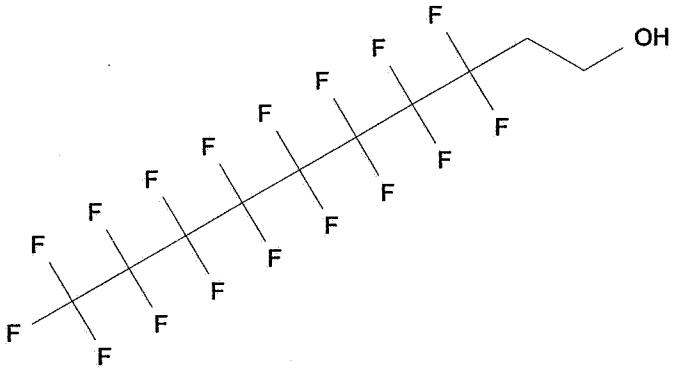
The purpose of the procedure is to provide a rapid screening method whereby substances that may adversely affect aerobic microorganisms from municipal sewage treatment plants can be identified and to indicate the suitable non-inhibitory concentrations of test substances to be used in biodegradability tests. The test uses a microbial inoculum and a synthetic sewage feed. The respiration rate of the test system is measured after an exposure period of 30 minutes under controlled laboratory conditions. The inhibitory effect of the test substance at the particular concentrations is expressed as percentage of the mean respiration rate of two controls.

3.2 Test Components

3.2.1 Chemical System

3.2.1.1 Test Substance

Name:	Telomer B 8-2 Alcohol
Synonym:	(Perfluorooctyl)ethanol
Active substance(s)	Telomer B 8-2 Alcohol, 99%
CAS Name:	1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-
CAS Number(s):	678-39-7
Structure:	



H Number	H-24691
Lot Number:	P.00/001
EMSE Sample Number:	E93386-63
Concentration of a.s., nominal:	99%
Concentration of a.s., analyzed:	99.2%
Certificate of Analysis Date:	13-Sept-2001
Date Received:	26-Mar-2002
Solubility at 25°C:	~140 $\mu g\ L^{-1}$
Vapor pressure:	0.023 mm Hg
Stability:	Stable at ambient room temperature
Appearance/Color:	White solid
Storage Conditions:	Room temperature; keep tightly closed
Safety Precautions:	Wear lab coat, protective gloves, and safety glasses

3.2.1.2 *Reference Substance*

Toxic Reference

Name:	3,5-dichlorophenol
Active substance(s)	3,5-dichlorophenol
CAS Number(s):	591-35-5
Product Number:	Aldrich D70600
Lot Number:	15809KI
EMSE Sample No.	M57657
Concentration of a.s., nominal:	97%
Concentration of a.s., analyzed:	99.7%
Certificate of Analysis Date:	August 2000
Expiry Date:	August 2005
Date Received:	January 2002
Appearance/Color:	White Powder
Storage Conditions:	Store in a cool dry place; keep tightly closed
Safety Precautions:	Irritant; wear suitable gloves and eye/face protection.
Manufacturer:	Aldrich Chemical Co., Inc.
Manufacturer Address:	1001 West St. Paul Milwaukee, WI 53233 USA

3.2.1.3 *Test Vehicle*

The test substance was dissolved in a stock solution of ethanol at 10000, 3000, 1000, 300, and 100 mg L^{-1}.

3.2.1.4 *Application Information*

The test substance was added to the test vessels at the following concentrations: 10000, 3200, 1000, 320 and 100 µg L^{-1}.

3.2.2 *Biological System*

Secondary activated sludge from Wilmington, DE USA Publically Owned Treatment Works (POTW) was used as the microbial inoculum. The activated sludge was aerated and used within 24 h of collection. The sludge was washed as follows: first centrifuge the sludge for about 10 min at approximately 10,000 m s^{-2} and discard the supernatant. Resuspend the sludge in tap water, remove this by recentrifuging, and then repeat twice more. An aliquot of the final sludge suspension was weighed, dried and the ratio of wet sludge to its dry weight determined. Based on this ratio, calculated amounts of wet sludge were suspended in tap water to yield a concentration equivalent to about 2 to 4 g per liter (on dry weight basis).

3.2.3 *Physical System*

3.2.3.1 *Test Units*

All test vessels are 300-mL Biological Oxygen Demand (BOD) glass bottles for incubation and Dissolved Oxygen (DO) determinations.

3.2.3.2 *Test Conditions*

Test solutions were kept closed and not aerated during a 30-minute incubation period at room temperature.

3.3 *Test Conduct*

Sufficient microbial solution for all the test vessels except the negative control was made. The microbial solution contained 32 mL of synthetic sewage feed, 400 mL microbial inoculum (containing 2 to 4 g dry weight/L), and about 568 mL tap water to make one L. The microbial solution was aerated at about 0.5 to 1 liter per minute until used. The test was initiated with the first positive control by adding 300 mL of the microbial solution to the test vessel and the test vessel stoppered. Aeration was stopped. The test vessels were continuously stirred using a stir bar and stir plate. After approximately 15 minutes (an arbitrary but convenient interval) the above procedure was repeated, except that the solvent was added to the test vessel at the concentration that was used with the test substance (first solvent control) and the was test initiated by adding 300 mL of the microbial solution to the test vessel. The test vessel was immediately capped. Again, after approximately 15 minutes this procedure was repeated, this time with a known amount of the reference substance with no solvent first added to the test vessel and the test initiated by adding 300 mL of the microbial solution to the test vessel and capping it. The procedure was repeated at approximately 15-minute intervals with the reference substance and then the test substance in solvent to give a series of test vessels containing different concentrations of the test substance. The negative control (sec. 12.7) test was performed next and then the second solvent control was performed. The final flask was a second positive control, prepared exactly as the first.

The test was conducted so that the reference and test substances were bracketed by the two controls, for example:

1. first positive control
2. first solvent control
3. reference substance (no solvent) at 3 concentrations
4. test substance (with solvent) at 5 concentrations
5. negative (abiotic) control at highest concentration of test substance
6. second solvent
7. second positive control

3.4 *Parameters Observed*

3.4.1 *Analysis of Test Systems*

The respiration rate of a well-mixed sample of each treatment was determined after exactly 30 minutes incubation time with no further aeration from the beginning of the 30-minute incubation period once the microbial solution had been added to the test vessel. The oxygen

concentration was measured with an oxygen electrode and recorded over a period of about 10 minutes. During measurement, the samples were continuously stirred with the built-in stirrer and measurements recorded using the attached printer. The rate of rate of oxygen consumption (in mg O_2 L^{-1} h^{-1}) was determined from the most linear part of the respiration curve over a period of about 10 minute.

The water temperature was measured in the positive control medium at the start and the end of the incubation period.

3.4.2 *Analysis of the Test Substance Stock Solutions*

Concentrations of the test substance stock solutions were estimated by GC/MS.

GC/MS Conditions for Telomer B 8-2 Alcohol Analysis

Gas Chromotography:	Hewlett Packard (HP) 5890 Series II
Dectector:	HP 5972 Series Mass Selective Detector
GC Column:	Restek Rtx®-200; 60 m, 0.25 mm ID, 1 µm film thickness
Mobile Phase:	Helium
Flow Rate:	1.4 mL/min (constant flow mode)
Column Temperature:	80°C for 1 min. 15°C/min to 300°C. Hold 10 minutes
Injection Volume:	1 µL splitless injection
Ionization mode:	Electron Ionization
Data acquisition mode:	selected ion monitoring, acquiring *m/z* of 31, 69, 95, 131, 463

3.5 ***Result Analysis***

3.5.1 *Respiration Rate*

The respiration rate was calculated from the recorder output as mg O_2 L^{-1} h^{-1} over a 10 min period. This can be done by graphing mg O_2 L^{-1} on the y-axis and time in hours on the x-axis, drawing the line of best fit, and then determining the slope of the line. The slope of the line is the extent to which the y-axis changes for 1 unit of change in the x-axis. Alternatively, the respiration rate can be calculated as follows:

$$b = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2}$$

where

b = Respiration Rate
n = pairs of x and y values
x = time in hours
y = mg O_2 L^{-1} at time = x

The portion of the respiration curve over which the respiration rate is measured should be linear.

3.5.2 *Inhibitory effect*

The inhibitory effects was determined by comparing the rates at each concentration of either test or reference substance to the rates in the controls. The results are expressed as percentage of the mean value of the respiration rates of the two controls according to:

$$\left[1 - \frac{2R_S}{R_{c1} + R_{c2}}\right] * 100 = \text{Percent inhibition}$$

where

Rs = oxygen consumption rate at tested concentration of test substance

R_{c1} = oxygen consumption rate of positive control 1

R_{c2} = oxygen consumption rate of positive control 2

The 30-minute EC_{50} (Effective Concentration of the substance giving a calculated or interpolated inhibition of oxygen consumption of 50% compared with a blank control) and their 95%-confidence limits are calculated by standard statistical methods. If possible, the EC_{20} and the EC_{80} also will be calculated and reported for the test substance.

The percent inhibition is calculated at each test concentration as above. For the test substance, the percent inhibition is plotted against concentration as a log-normal (or log-probability) graph and an EC_{50} value derived from the graph. For the reference substance, the percent inhibition is plotted against concentration as a normal-normal graph and an EC_{50} value derived directly from the graph.

3.6 *Validity Criteria of the Study*

The test results are valid if:

- The two positive control respiration rates (PCRR) and the two solvent control respiration rates are within 15 percent of each other:
- The EC_{50} (30 minutes) of 3,5-dichlorophenol is in the accepted range of 5 to 30 mg L^{-1}, which is determined by graphing the percent inhibition on the y-axis and the concentration of the reference substance at which that level of inhibition was measured on the x-axis. The EC_{50} can be determined directly from the graph.

3.7 *Protocol Deviations*

The test systems were not maintained at a temperature of 23 ± 2°C. This did not affect the test, however, because the validity criteria of the test were met, as stated in section 3.6.

4.0 RESULTS AND DISCUSSION

- EC_{50} of the test substance at 30 min was greater than 10000 µg test substance per liter
- EC_{20} of the test substance at 30 min was greater than 10000 µg test substance per liter
- EC_{80} of the test substance at 30 min was greater than 10000 µg test substance per liter

The test substance showed no acute toxic effect towards the microbial inoculum (activated sludge) at an oxygen consumption inhibition level $\geq$ 20% at the highest concentration of 10000 µg L^{-1} that was tested.

- EC_{50} of the reference substance at 30 min was 12 mg reference substance per liter, which was within the acceptable range of from 5 to 30 mg L^{-1}

The two positive control respiration rates were within 40% of each other and not within 15% as required for a valid test. The test is valid, however, because the reference substance has an EC_{50} of 12 mg L^{-1}, which is within the acceptable range. Additionally, if the first positive control values is not used to calculate the respiration inhibition because of the low temperature at which it was studied, the EC_{50} of the reference substance would be 19 mg L^{-1}, which is also within the acceptable range. All microbial inoculum was refrigerated overnight and was not allowed to completely warm to room temperature prior to the start of the experiment. This resulted in the temperature of the first positive control of 15.0°C, which was signficantly lower than the temperature of the second positive control solution of 20.2°C. On the other hand, the respiration rates of the two solvent control solutions, which were used to calculate the respiration inhibition of the test substance, were within 9% of each other. This is less than 15% as required for a valid test.

Temperatures in the positive control medium at the beginning and end of the incubation period were 15.0 and 20.2°C, respectively.

The concentrations of the test substance stock solutions were determined to be 10910, 3450, 1284, 320, and 100 mg L^{-1} (Table 2). This compares favorably to the target concentrations of 10000, 3000, 1000, 300, and 100 mg L^{-1}, respectively, for test substance in the stock solutions.

5.0 CONCLUSIONS

- The EC_{50} of the test substance is estimated to be greater than 10000 µg L^{-1} (10 mg L^{-1})
- The EC_{50} of the reference substance is 12 mg L^{-1}
- The test is valid.

6.0 RETENTION OF RECORDS

For the periods demanded by GLP guidelines and specific country requirements, study documents and materials will be archived at DuPont CR&D Environmental & Microbiological Sciences and Engineering (EMSE) and/or Iron Mountain, Wilmington, Delaware USA, including but not limited to:

- study protocol;
- any protocol and/or report amendments or addenda or SOP deviations;
- all raw data;
- one original signed copy of the final report;
- laboratory-specific or site-specific raw data such as personnel files, instrument, equipment, refrigerator, and/or freezer raw data.

Documents and materials are archived according to the principles of Good Laboratory Practice in the organization of the testing facility.

7.0 DISPOSAL OF TEST SUBSTANCE

After issuance of the final report, the remaining test substance will be stored at the DuPont EMSE laboratory until testing is complete and then destroyed by incineration, unless other arrangements are made between the submitter and the Test Facility.

8.0 REFERENCES

1. OECD (1984) Guideline for Testing of Chemicals, Section 2, No. 209: "Activated Sludge, Respiration Inhibition Test", adopted April 04, 1984.
2. International Standard ISO 8192: "Water quality – Test for inhibition of oxygen consumption by activated sludge". First edition 1986-07-15. Ref. No. ISO 8192-1986 (E).

TABLE 1
INFLUENCE OF TEST SUBSTANCE TELOMER B 8-2 ALCOHOL AND REFERENCE SUBSTANCE 3, 5-DICHLOROPHENOL ON THE O_2 CONSUMPTION OF ACTIVATED SLUDGE

No.	Treatment	Concentration	O_2 Concentration†		O_2 Consumption	Inhibition‡
			Start	End		
		mg/L	*mg O_2/L*		*mg O_2/L/h*	*%*
1	Positive Control 1	0	7.17	6.75	2.89	NA¶
13	Positive Control 2	0	6.51	5.80	4.33	NA
	Mean				3.61	
	Standard deviation				1.02	
2	Solvent Control 1	0	6.74	5.80	5.91	NA
12	Solvent Control 2	0	5.62	4.61	6.46	NA
	Mean				6.18	
	Standard deviation				0.39	
3	3,5-dicholorophenol	16	8.90	8.73	1.12	69.0
4	3,5-dicholorophenol	5	8.07	7.67	2.66	26.2
5	3,5-dicholorophenol	1.6	7.68	7.11	3.78	-0.85
6	Telomer B 8-2 Alcohol	10	5.96	4.82	7.16	-15.8
7	Telomer B 8-2 Alcohol	3.2	5.79	4.62	7.46	-19.7
8	Telomer B 8-2 Alcohol	1.0	5.68	4.50	7.23	-16.9
9	Telomer B 8-2 Alcohol	0.32	5.71	4.65	6.72	-8.71
10	Telomer B 8-2 Alcohol	0.10	5.64	4.57	7.70	-24.5
11	Telomer B 8-2 Alcohol negative control	10	9.86	9.80	0.51	91.7

†The readings of the Dissolved O_2 meter at start and end of 10-minute period over which data was collected after 30 min of aeration

‡Inhibition of the reference substance was calculated using the average value of the positive control solutions; the inhibition of the test substance was calculated using an average value of the solvent control solutions.

¶ NA = Not Applicable.

TABLE 2
CONCENTRATIONS OF THE TEST SUBSTANCE STOCK SOLUTIONS

EMSE Number	Desired Stock Solution Concentrations	Measured Stock Solution Concentrations
	mg L^{-1}	*mg L*$^{-1}$
M57777	10 000	10 910
M57778	3 000	3450
M57779	1000	1284
M57780	300	320
M57781	100	100

FIGURE 1
INFLUENCE OF THE REFERENCE SUBSTANCE 3,5-DICHLOROPHENOL ON THE RESPIRATION RATE OF AEROBIC WASTEWATER MICROORGANISMS AFTER 30 MINUTES OF EXPOSURE

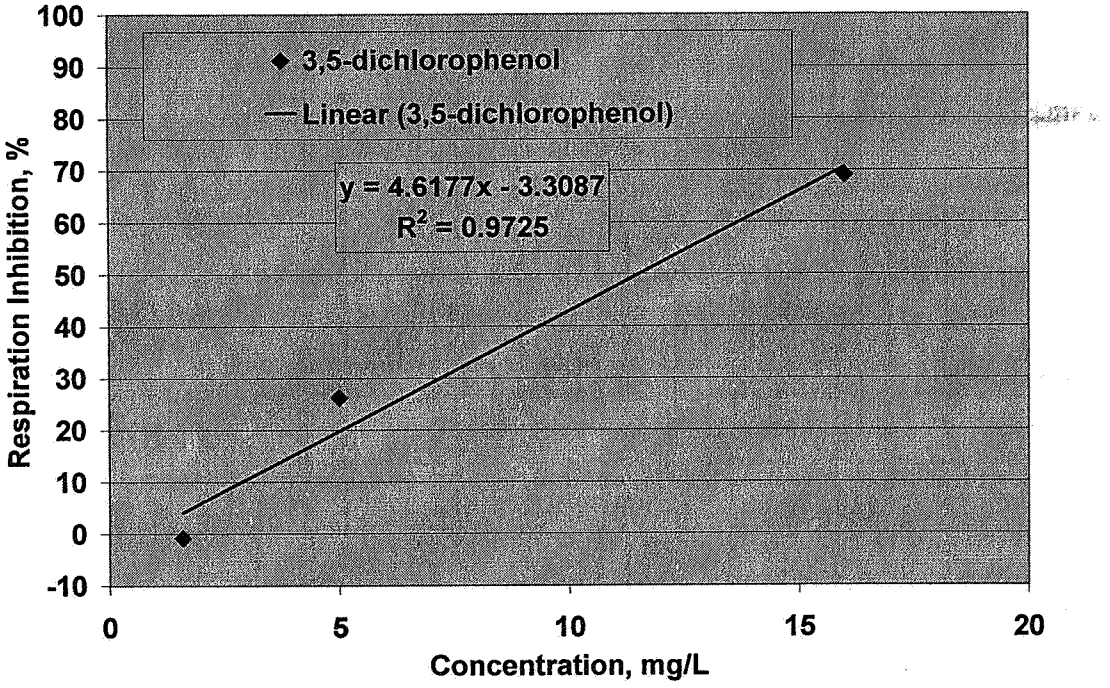
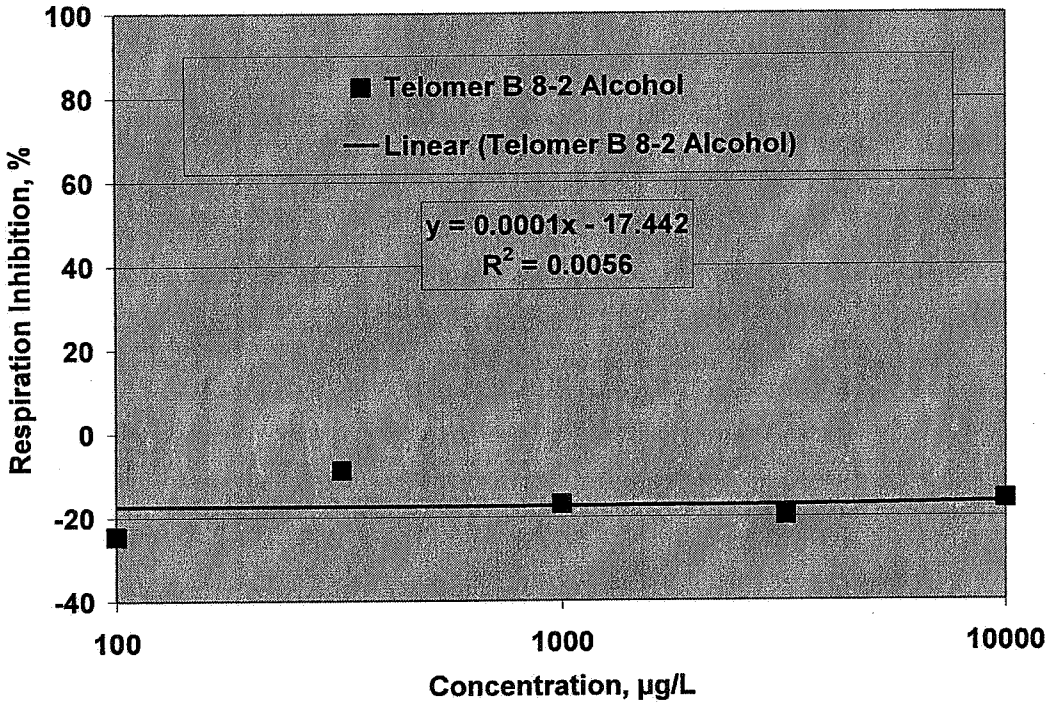


FIGURE 2
INFLUENCE OF THE TEST SUBSTANCE TELOMER B 8-2 ALCOHOL ON THE RESPIRATION RATE OF AEROBIC WASTEWATER MICROORGANISMS AFTER 30 MINUTES OF EXPOSURE



APPENDIX A
DATA TABLES AND CALCULATIONS FOR POSITIVE CONTROLS, SOLVENT CONTROLS, REFERENCE AND TEST SUBSTANCES, AND NEGATIVE CONTROL SAMPLES

Positive Control 1

Positive Control 1

Rate of Respiration (R_{c1}) = 2.894351 mg O_2 / L / h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	9:03:05	7.17	0:00:00	0	0.00	0.00
2	9:03:35	7.20	0:00:30	30	0.50	0.01
3	9:04:04	7.17	0:00:59	59	0.98	0.02
4	9:04:35	7.14	01:30.0	90	1.50	0.03
5	9:05:05	7.13	0:02:00	120	2.00	0.03
6	9:05:34	7.10	0:02:29	149	2.48	0.04
7	9:06:04	7.08	0:02:59	179	2.98	0.05
8	9:06:35	7.05	0:03:30	210	3.50	0.06
9	9:07:04	7.02	0:03:59	239	3.98	0.07
10	9:07:34	7.00	0:04:29	269	4.48	0.07
11	9:08:05	6.97	0:05:00	300	5.00	0.08
12	9:08:35	6.95	0:05:30	330	5.50	0.09
13	9:09:04	6.93	0:05:59	359	5.98	0.10
14	9:09:34	6.89	0:06:29	389	6.48	0.11
15	9:10:04	6.87	0:06:59	419	6.98	0.12
16	9:10:34	6.85	0:07:29	449	7.48	0.12
17	9:11:05	6.83	0:08:00	480	8.00	0.13
18	9:11:34	6.81	0:08:29	509	8.48	0.14
19	9:12:04	6.77	0:08:59	539	8.98	0.15
20	9:12:34	6.75	0:09:29	569	9.48	0.16

Positive Control 2

Positive Control 2

Rate of Respiration (R_{c2}) = 4.331447 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	12:01:32	6.51	0:00:00	0	0.00	0.00
2	12:02:03	6.44	0:00:31	31	0.52	0.01
3	12:02:32	6.41	0:01:00	60	1.00	0.02
4	12:03:02	6.37	0:01:30	90	1.50	0.03
5	12:03:33	6.34	0:02:01	121	2.02	0.03
6	12:04:03	6.33	0:02:31	151	2.52	0.04
7	12:04:32	6.26	0:03:00	180	3.00	0.05
8	12:05:03	6.24	0:03:31	211	3.52	0.06
9	12:05:33	6.20	0:04:01	241	4.02	0.07
10	12:06:02	6.15	0:04:30	270	4.50	0.08
11	12:06:33	6.12	0:05:01	301	5.02	0.08
12	12:07:03	6.08	0:05:31	331	5.52	0.09
13	12:07:32	6.05	0:06:00	360	6.00	0.10
14	12:08:02	6.01	0:06:30	390	6.50	0.11
15	12:08:32	5.98	0:07:00	420	7.00	0.12
16	12:09:02	5.95	0:07:30	450	7.50	0.13
17	12:09:32	5.92	0:08:00	480	8.00	0.13
18	12:10:03	5.88	0:08:31	511	8.52	0.14
19	12:10:33	5.84	0:09:01	541	9.02	0.15
20	12:11:03	5.80	0:09:31	571	9.52	0.16

Solvent Control 1

Solvent Control 1

Rate of Respiration () = 5.911737 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Time	Time	Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	9:18:34	6.74	0:00:00	0	0.00	0.00
2	9:19:05	6.70	0:00:31	31	0.52	0.01
3	9:19:34	6.65	0:01:00	60	1.00	0.02
4	9:20:04	6.59	0:01:30	90	1.50	0.03
5	9:20:35	6.55	0:02:01	121	2.02	0.03
6	9:21:05	6.50	0:02:31	151	2.52	0.04
7	9:21:35	6.46	0:03:01	181	3.02	0.05
8	9:22:04	6.40	0:03:30	210	3.50	0.06
9	9:22:34	6.35	0:04:00	240	4.00	0.07
10	9:23:04	6.30	0:04:30	270	4.50	0.08
11	9:23:35	6.26	0:05:01	301	5.02	0.08
12	9:24:04	6.20	0:05:30	330	5.50	0.09
13	9:24:35	6.15	0:06:01	361	6.02	0.10
14	9:25:04	6.10	0:06:30	390	6.50	0.11
15	9:25:34	6.05	0:07:00	420	7.00	0.12
16	9:26:04	6.00	0:07:30	450	7.50	0.13
17	9:26:35	5.96	0:08:01	481	8.02	0.13
18	9:27:04	5.94	0:08:30	510	8.50	0.14
19	9:27:35	5.85	0:09:01	541	9.02	0.15
20	9:28:04	5.80	0:09:30	570	9.50	0.16

Solvent Control 2

Solvent Control 2

Rate of Respiration ($_2$) = 6.458075 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Time	Time	Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	11:47:46	5.62	0:00:00	0	0.00	0.00
2	11:48:15	5.57	0:00:29	29	0.48	0.01
3	11:48:45	5.52	0:00:59	59	0.98	0.02
4	11:49:15	5.46	0:01:29	89	1.48	0.02
5	11:49:46	5.40	0:02:00	120	2.00	0.03
6	11:50:16	5.35	0:02:30	150	2.50	0.04
7	11:50:46	5.30	0:03:00	180	3.00	0.05
8	11:51:15	5.23	0:03:29	209	3.48	0.06
9	11:51:45	5.19	0:03:59	239	3.98	0.07
10	11:52:15	5.13	0:04:29	269	4.48	0.07
11	11:52:46	5.08	0:05:00	300	5.00	0.08
12	11:53:15	5.03	0:05:29	329	5.48	0.09
13	11:53:45	4.98	0:05:59	359	5.98	0.10
14	11:54:16	4.93	0:06:30	390	6.50	0.11
15	11:54:45	4.87	0:06:59	419	6.98	0.12
16	11:55:15	4.81	0:07:29	449	7.48	0.12
17	11:55:45	4.75	0:07:59	479	7.98	0.13
18	11:56:15	4.70	0:08:29	509	8.48	0.14
19	11:56:46	4.65	0:09:00	540	9.00	0.15
20	11:57:15	4.61	0:09:29	569	9.48	0.16

Reference 1: 16 mg L^{-1} 3,5-dichlorophenol

Reference 1: 16 mg/L 3,5-dichlorophenol

Rate of Respiration (R_a) = 1.11818 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	9:33:40	8.90	0:00:00	0	0.00	0.00
2	9:34:11	8.91	0:00:31	31	0.52	0.01
3	9:34:40	8.90	0:01:00	60	1.00	0.02
4	9:35:10	8.89	0:01:30	90	1.50	0.03
5	9:35:41	8.88	0:02:01	121	2.02	0.03
6	9:36:10	8.86	0:02:30	150	2.50	0.04
7	9:36:40	8.86	0:03:00	180	3.00	0.05
8	9:37:11	8.85	0:03:31	211	3.52	0.06
9	9:37:40	8.83	0:04:00	240	4.00	0.07
10	9:38:10	8.83	0:04:30	270	4.50	0.08
11	9:38:40	8.83	0:05:00	300	5.00	0.08
12	9:39:10	8.82	0:05:30	330	5.50	0.09
13	9:39:41	8.81	0:06:01	361	6.02	0.10
14	9:40:11	8.80	0:06:31	391	6.52	0.11
15	9:40:41	8.79	0:07:01	421	7.02	0.12
16	9:41:10	8.77	0:07:30	450	7.50	0.13
17	9:41:41	8.76	0:08:01	481	8.02	0.13
18	9:42:11	8.76	0:08:31	511	8.52	0.14
19	9:42:40	8.74	0:09:00	540	9.00	0.15
20	9:43:10	8.73	0:09:30	570	9.50	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 1.1182}{2.89435 + 4.3314} \times 100 = 69.05\ \%$$

Reference 2: 5 mg L^{-1} 3,5-dichlorophenol

Reference 2: 5 mg/L 3,5-dichlorophenol

Rate of Respiration (R_a) = 2.665095 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	9:47:52	8.07	0:00:00	0	0.00	0.00
2	9:48:23	8.08	0:00:31	31	0.52	0.01
3	9:48:53	8.06	0:01:01	61	1.02	0.02
4	9:49:22	8.04	0:01:30	90	1.50	0.03
5	9:49:52	8.02	0:02:00	120	2.00	0.03
6	9:50:23	7.99	0:02:31	151	2.52	0.04
7	9:50:53	7.98	0:03:01	181	3.02	0.05
8	9:51:23	7.95	0:03:31	211	3.52	0.06
9	9:51:53	7.93	0:04:01	241	4.02	0.07
10	9:52:22	7.90	0:04:30	270	4.50	0.08
11	9:52:52	7.88	0:05:00	300	5.00	0.08
12	9:53:22	7.86	0:05:30	330	5.50	0.09
13	9:53:52	7.83	0:06:00	360	6.00	0.10
14	9:54:22	7.80	0:06:30	390	6.50	0.11
15	9:54:53	7.79	0:07:01	421	7.02	0.12
16	9:55:23	7.76	0:07:31	451	7.52	0.13
17	9:55:53	7.76	0:08:01	481	8.02	0.13
18	9:56:22	7.72	0:08:30	510	8.50	0.14
19	9:56:53	7.70	0:09:01	541	9.02	0.15
20	9:57:23	7.67	0:09:31	571	9.52	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 2.6651}{2.89435 + 4.3314} \times 100 = 26.23\ \%$$

Reference 3: 1.6 mg L^{-1} 3,5-dichlorophenol

Reference 3: 1.6 mg/L 3,5-dichlorophenol

Rate of Respiration (R_s) = 3.643633 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	10:03:44	7.68	0:00:00	0	0.00	0.00
2	10:04:15	7.67	0:00:31	31	0.52	0.01
3	10:04:45	7.64	0:01:01	61	1.02	0.02
4	10:05:14	7.62	0:01:30	90	1.50	0.03
5	10:05:45	7.59	0:02:01	121	2.02	0.03
6	10:06:15	7.55	0:02:31	151	2.52	0.04
7	10:06:44	7.52	0:03:00	180	3.00	0.05
8	10:07:15	7.49	0:03:31	211	3.52	0.06
9	10:07:44	7.47	0:04:00	240	4.00	0.07
10	10:08:14	7.40	0:04:30	270	4.50	0.08
11	10:08:45	7.39	0:05:01	301	5.02	0.08
12	10:09:14	7.36	0:05:30	330	5.50	0.09
13	10:09:44	7.34	0:06:00	360	6.00	0.10
14	10:10:14	7.31	0:06:30	390	6.50	0.11
15	10:10:45	7.29	0:07:01	421	7.02	0.12
16	10:11:14	7.24	0:07:30	450	7.50	0.13
17	10:11:45	7.22	0:08:01	481	8.02	0.13
18	10:12:14	7.19	0:08:30	510	8.50	0.14
19	10:12:44	7.15	0:09:00	540	9.00	0.15
20	10:13:15	7.11	0:09:31	571	9.52	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 3.6436}{2.89435 + 4.3314} \times 100 = -0.85\ \%$$

Test 1: 10 000 µg L^{-1} Telomer B 8-2 Alcohol

Test 1: 10000 mg/L TBA

Rate of Respiration (R_s) = 7.163217 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	10:17:47	5.96	0:00:00	0	0.00	0.00
2	10:18:17	5.89	0:00:30	30	0.50	0.01
3	10:18:46	5.84	0:00:59	59	0.98	0.02
4	10:19:17	5.77	0:01:30	90	1.50	0.03
5	10:19:47	5.71	0:02:00	120	2.00	0.03
6	10:20:17	5.66	0:02:30	150	2.50	0.04
7	10:20:46	5.60	0:02:59	179	2.98	0.05
8	10:21:16	5.54	0:03:29	209	3.48	0.06
9	10:21:46	5.48	0:03:59	239	3.98	0.07
10	10:22:17	5.42	0:04:30	270	4.50	0.08
11	10:22:47	5.35	0:05:00	300	5.00	0.08
12	10:23:17	5.30	0:05:30	330	5.50	0.09
13	10:23:47	5.24	0:06:00	360	6.00	0.10
14	10:24:17	5.18	0:06:30	390	6.50	0.11
15	10:24:47	5.12	0:07:00	420	7.00	0.12
16	10:25:16	5.06	0:07:29	449	7.48	0.12
17	10:25:47	5.00	0:08:00	480	8.00	0.13
18	10:26:16	4.94	0:08:29	509	8.48	0.14
19	10:26:47	4.88	0:09:00	540	9.00	0.15
20	10:27:17	4.82	0:09:30	570	9.50	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 7.1632}{5.91174 + 6.4581} \times 100 = -15.82\ \%$$

Test 2: 3 200 µg L^{-1} Telomer B 8-2 Alcohol

Test 2: 3000 mg/L TBA

Rate of Respiration (R_a) = 7.403571 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	10:32:40	5.79	0:00:00	0	0.00	0.00
2	10:33:11	5.73	0:00:31	31	0.52	0.01
3	10:33:41	5.66	0:01:01	61	1.02	0.02
4	10:34:11	5.59	0:01:31	91	1.52	0.03
5	10:34:41	5.54	0:02:01	121	2.02	0.03
6	10:35:11	5.48	0:02:31	151	2.52	0.04
7	10:35:41	5.42	0:03:01	181	3.02	0.05
8	10:36:10	5.36	0:03:30	210	3.50	0.06
9	10:36:40	5.29	0:04:00	240	4.00	0.07
10	10:37:11	5.23	0:04:31	271	4.52	0.08
11	10:37:40	5.17	0:05:00	300	5.00	0.08
12	10:38:10	5.10	0:05:30	330	5.50	0.09
13	10:38:40	5.05	0:06:00	360	6.00	0.10
14	10:39:10	4.98	0:06:30	390	6.50	0.11
15	10:39:41	4.92	0:07:01	421	7.02	0.12
16	10:40:11	4.85	0:07:31	451	7.52	0.13
17	10:40:41	4.80	0:08:01	481	8.02	0.13
18	10:41:11	4.74	0:08:31	511	8.52	0.14
19	10:41:41	4.68	0:09:01	541	9.02	0.15
20	10:42:10	4.62	0:09:30	570	9.50	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 7.4036}{5.91174 + 6.4581} \times 100 = -19.70 \ \%$$

Test 3: 1 000 µg L^{-1} Telomer B 8-2 Alcohol

Test 3: 1000 mg/L TBA

Rate of Respiration (R_a) = 7.231915 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	10:47:39	5.68	0:00:00	0	0.00	0.00
2	10:48:09	5.60	0:00:30	30	0.50	0.01
3	10:48:38	5.54	0:00:59	59	0.98	0.02
4	10:49:08	5.47	0:01:29	89	1.48	0.02
5	10:49:38	5.40	0:01:59	119	1.98	0.03
6	10:50:08	5.34	0:02:29	149	2.48	0.04
7	10:50:39	5.28	0:03:00	180	3.00	0.05
8	10:51:08	5.23	0:03:29	209	3.48	0.06
9	10:51:39	5.17	0:04:00	240	4.00	0.07
10	10:52:09	5.11	0:04:30	270	4.50	0.08
11	10:52:38	5.05	0:04:59	299	4.98	0.08
12	10:53:08	4.99	0:05:29	329	5.48	0.09
13	10:53:39	4.93	0:06:00	360	6.00	0.10
14	10:54:09	4.88	0:06:30	390	6.50	0.11
15	10:54:39	4.81	0:07:00	420	7.00	0.12
16	10:55:09	4.75	0:07:30	450	7.50	0.13
17	10:55:39	4.69	0:08:00	480	8.00	0.13
18	10:56:09	4.64	0:08:30	510	8.50	0.14
19	10:56:38	4.58	0:08:59	539	8.98	0.15
20	10:57:09	4.50	0:09:30	570	9.50	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 7.2319}{5.91174 + 6.4581} \times 100 = -16.92845 \ \%$$

Test 4: 320 µg L^{-1} Telomer B 8-2 Alcohol

Test 4: 300 mg/L TBA

Rate of Respiration (R_s) = 6.723553 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
		mg/L	*s*	*s*	*m*	*h*
1	11:03:11	5.71	0:00:00	0	0.00	0.00
2	11:03:40	5.65	0:00:29	29	0.48	0.01
3	11:04:11	5.60	0:01:00	60	1.00	0.02
4	11:04:40	5.54	0:01:29	89	1.48	0.02
5	11:05:10	5.47	0:01:59	119	1.98	0.03
6	11:05:40	5.44	0:02:29	149	2.48	0.04
7	11:06:10	5.38	0:02:59	179	2.98	0.05
8	11:06:40	5.32	0:03:29	209	3.48	0.06
9	11:07:10	5.26	0:03:59	239	3.98	0.07
10	11:07:40	5.22	0:04:29	269	4.48	0.07
11	11:08:10	5.15	0:04:59	299	4.98	0.08
12	11:08:40	5.09	0:05:29	329	5.48	0.09
13	11:09:10	5.03	0:05:59	359	5.98	0.10
14	11:09:41	4.98	0:06:30	390	6.50	0.11
15	11:10:10	4.93	0:06:59	419	6.98	0.12
16	11:10:41	4.87	0:07:30	450	7.50	0.13
17	11:11:11	4.81	0:08:00	480	8.00	0.13
18	11:11:40	4.75	0:08:29	509	8.48	0.14
19	11:12:10	4.70	0:08:59	539	8.98	0.15
20	11:12:41	4.65	0:09:30	570	9.50	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 6.7236}{5.91174 + 6.4581} \times 100 = -8.71\ \%$$

Test 5: 100 µg L^{-1} Telomer B 8-2 Alcohol

Test 5: 100 mg/L TBA

Rate of Respiration (R_s) = 7.70189 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	11:20:42	5.64	0:00:00	0	0.00	0.00
2	11:21:12	5.32	0:00:30	30	0.50	0.01
3	11:21:42	4.91	0:01:00	60	1.00	0.02
4	11:22:12	4.60	0:01:30	90	1.50	0.03
5	11:22:41	4.36	0:01:59	119	1.98	0.03
6	11:23:11	4.20	0:02:29	149	2.48	0.04
7	11:23:41	4.11	0:02:59	179	2.98	0.05
8	11:24:11	4.02	0:03:29	209	3.48	0.06
9	11:24:42	3.96	0:04:00	240	4.00	0.07
10	11:25:12	3.89	0:04:30	270	4.50	0.08
11	11:25:41	3.84	0:04:59	299	4.98	0.08
12	11:26:12	3.79	0:05:30	330	5.50	0.09
13	11:26:42	3.75	0:06:00	360	6.00	0.10
14	11:27:11	3.72	0:06:29	389	6.48	0.11
15	11:27:41	3.69	0:06:59	419	6.98	0.12
16	11:28:12	3.65	0:07:30	450	7.50	0.13
17	11:28:42	3.61	0:08:00	480	8.00	0.13
18	11:29:12	3.58	0:08:30	510	8.50	0.14
19	11:29:41	4.43	0:08:59	539	8.98	0.15
20	11:30:11	4.57	0:09:29	569	9.48	0.16

$$\text{Percent Inhibition} = 1 - \frac{2 \times 7.7019}{5.91174 + 6.4581} \times 100 = -24.53\ \%$$

Negative control: Negative control with 10 000 µg L^{-1} Telomer B 8-2 Alcohol

Negative Control with TBA at 10000 mg/L

Rate of Respiration (R_s) = 0.51422 mg O_2 / L /h

Reading Number	Time of Day	DO Reading	Time	Total Time	Total Time	Total Time
	h:mm:ss	*mg/L*	*h:mm:ss*	*s*	*m*	*h*
1	11:34:30	9.86	0:00:00	0	0.00	0.00
2	11:35:01	9.87	0:00:31	31	0.52	0.01
3	11:35:30	9.88	0:01:00	60	1.00	0.02
4	11:36:01	9.89	0:01:31	91	1.52	0.03
5	11:36:30	9.89	0:02:00	120	2.00	0.03
6	11:37:01	9.88	0:02:31	151	2.52	0.04
7	11:37:31	9.87	0:03:01	181	3.02	0.05
8	11:38:00	9.87	0:03:30	210	3.50	0.06
9	11:38:31	9.86	0:04:01	241	4.02	0.07
10	11:39:00	9.85	0:04:30	270	4.50	0.08
11	11:39:30	9.85	0:05:00	300	5.00	0.08
12	11:40:00	9.85	0:05:30	330	5.50	0.09
13	11:40:30	9.78	0:06:00	360	6.00	0.10
14	11:41:00	9.85	0:06:30	390	6.50	0.11
15	11:41:31	9.84	0:07:01	421	7.02	0.12
16	11:42:00	9.82	0:07:30	450	7.50	0.13
17	11:42:30	9.82	0:08:00	480	8.00	0.13
18	11:43:01	9.81	0:08:31	511	8.52	0.14
19	11:43:30	9.82	0:09:00	540	9.00	0.15
20	11:44:01	9.80	0:09:31	571	9.52	0.16

$$\text{Percent Inhibition} = 1 - \frac{2x}{5.91174 + 6.4581} \times 100 = 91.68589\ \%$$