Customer Report

Biodegradability Testing

Project ID *0321-EAP-01-1*

Project Initiation Date 3/31/2021

prepared for:

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Table of Contents

Compliance Statement

Testing is conducted according to the required criteria established for ISO 17025 Accredited laboratories. The laboratory is independently audited, verifying this compliance.

This report is governed by and incorporates by reference, the conditions of testing as posted on the date of issuance, and is intended for the identified Project Owners exclusive use. This report sets forth our findings solely with respect to test samples identified herein. The results set forth in this report are not indicative or representative of the quality or characteristics of the lot from which a test sample was taken or any similar identical product unless specifically and expressly noted.

ISO 17025 Confidentiality

The lab shall be responsible through legally enforceable commitments for the management of all information obtained during the performance of lab activities.

The Project Owner will be contacted for approval in writing if the laboratory is requested to provide any details regarding the project, or project documentation.

Abstract

A study was conducted to determine the biological degradation of the provided test substance according to the ISO *14851 Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer*. The ISO *14851* method is designed to provide a screening of plastics for biodegradability in an aerobic aqueous medium. Total oxygen is either calculated based on the molecular formula (ThOD) or by measuring the amount of oxidizable matter in the test sample using a chemical oxygen demand (COD) determination. Testing is typically conducted for up to 60 days unless extended by request of the project owner.

At the end of the test time frame, the level of degradation is determined, with a plateau in the rate for oxygen consumption being indicative of achieving the maximum level for biodegradation of the sample in this test scheme.

Results and Discussion

Results are provided in the Result Data Tables.

Sample 1 - The test substance did achieve a degradation plateau at day 95 of testing as determined by a non-linear curve fit of the data for oxygen consumption. Consumption of O2 by the test system indicates that the test sample biodegraded to 34% of the ThOD following 95 days of testing.

Sample 2 - The test substance did achieve a degradation plateau at day 85 of testing as determined by a non-linear curve fit of the data for oxygen consumption. Consumption of O2 by the test system indicates that the test sample biodegraded to 18% of the ThOD following 85 days of testing.

Report Result Tables

Sample List

Sample #	Method Name	Sample Name	Sample Notes
Project - Images			
1		Hydropol 30164P	
2		Hydropol 33104P	
ISO 14851 - Standard for Determination of Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium - Method by Measuring the Oxygen Demand in a Closed Respirometer			
1		Hydropol 30164P	
2		Hydropol 33104P	
4		Reference Material - Aniline	

Test Method **ISO 14851 - Standard for Determination of Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium - Method by Measuring the Oxygen Demand in a Closed Respirometer**

Sample # 1 **Hydropol 30164P**

	Interval	Result
Inoculum: ***Environmental Culture (activated sludge) ()***		
Notes Section		
biodegradation plateau	95	27 % ThOD
Carbon concentration in test sample	0	55.6 %TC
Test sample concentration	0	72.5 mg/L COD
Final pH	95	6.9 pH
Final bacterial concentration	95	121000 CFU/ml

Sample # 2 **Hydropol 33104P**

	Interval	Result
Inoculum: ***Environmental Culture (activated sludge) ()***		
Notes Section		
biodegradation plateau	95	18 % ThOD
Carbon concentration in test sample	0	55.6 %TC
Test sample concentration	0	72.5 mg/L COD
Final pH	95	7 pH
Final bacterial concentration	95	145000 CFU/ml

Sample # 4 **Reference Material - Aniline**

	Interval	Result
Inoculum: ***Environmental Culture (activated sludge) ()***		
Notes Section		
biodegradation plateau	77	98 % ThOD
Carbon concentration in test sample	0	55.6 %TC
Test sample concentration	0	72.5 mg/L COD
Final pH	95	7.1 pH
Final bacterial concentration	95	180000 CFU/ml

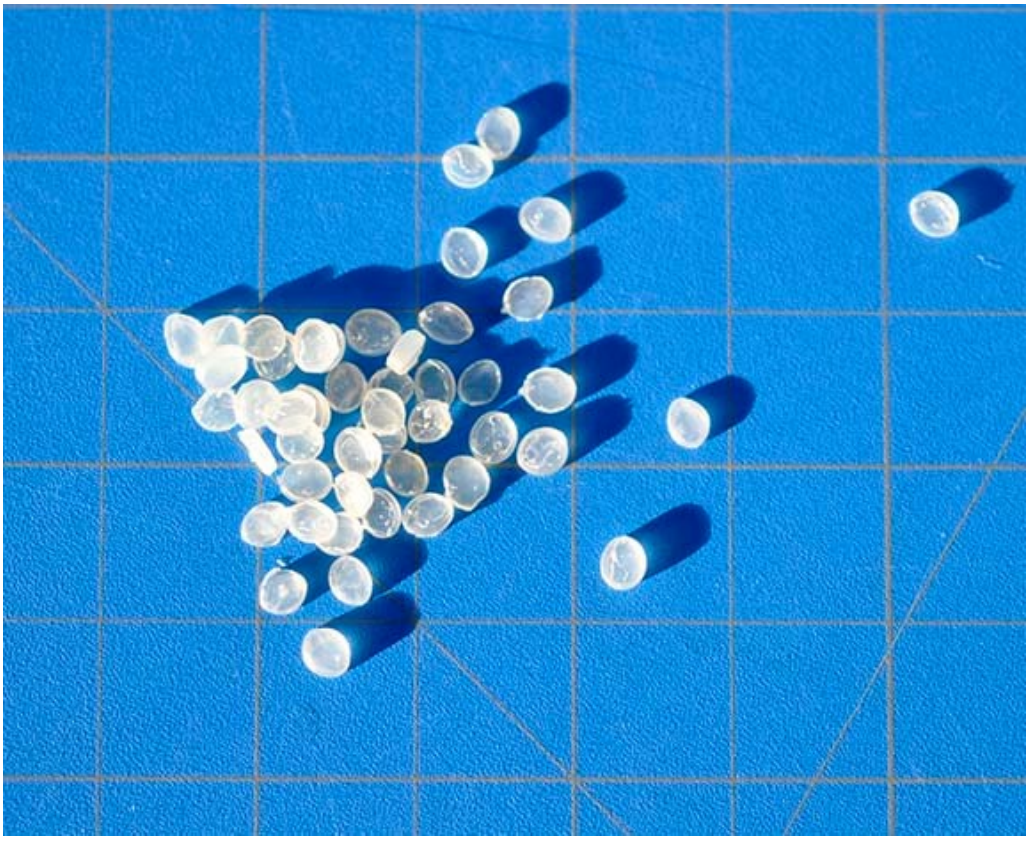
Report Image

Sample # 1 **Hydropol 30164P**

Test Method **Project - Images**

Inoculum ***None***

Image: **sample** ***Timepoint:*** **time - 0**



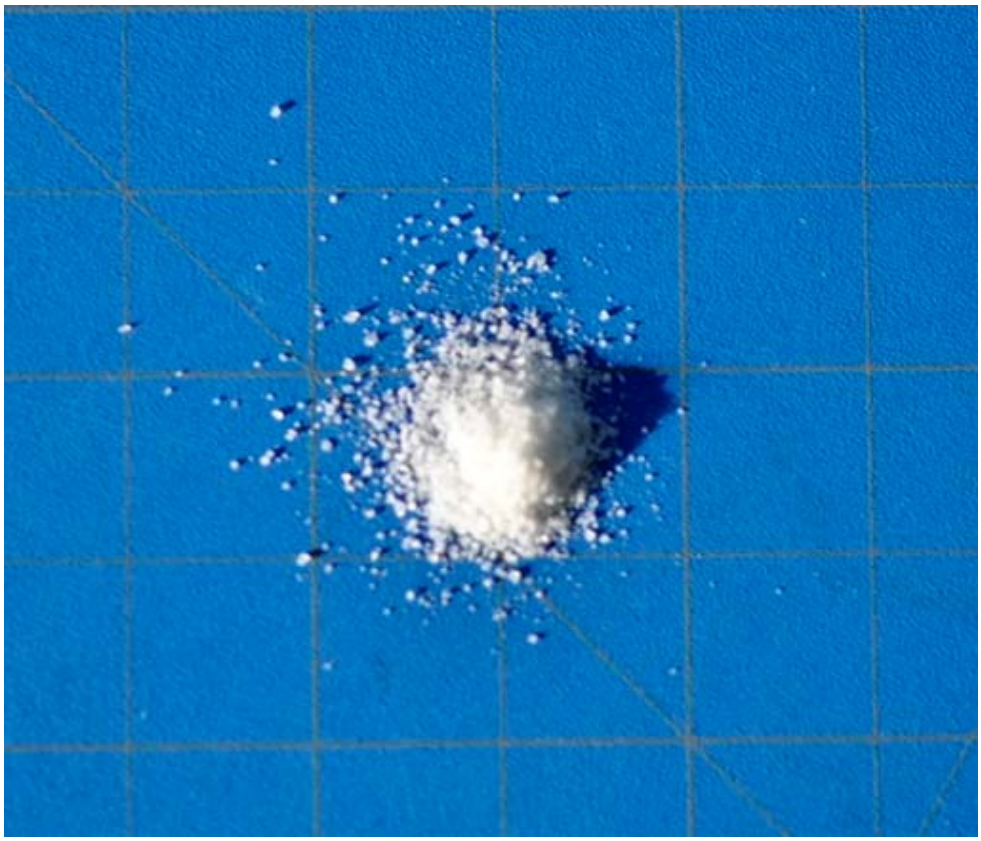
Report Image

Sample # 1 **Hydropol 30164P**

Test Method **Project - Images**

Inoculum ***None***

Image: **cryo-milled sample** ***Timepoint:*** **time - 0**



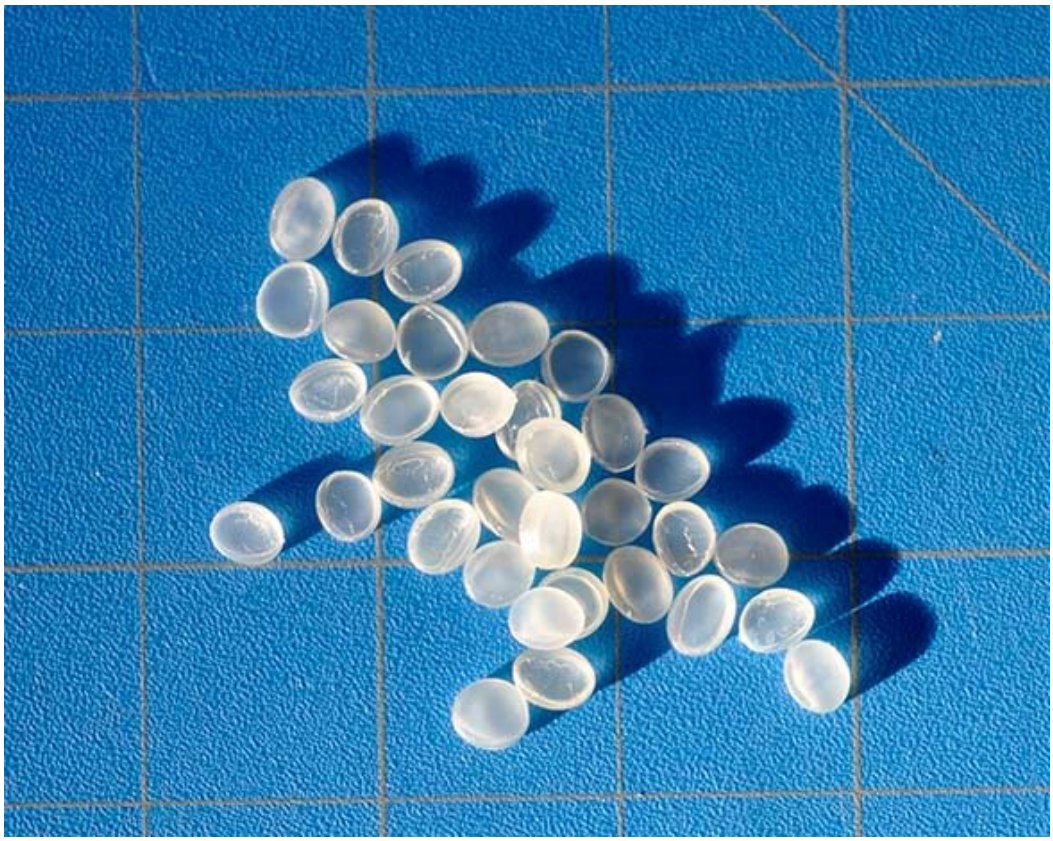
Report Image

Sample # 2 **Hydropol 33104P**

Test Method **Project - Images**

Inoculum ***None***

Image: **sample** ***Timepoint:*** **time - 0**



Report Image

Sample # 2 **Hydropol 33104P**

Test Method **Project - Images**

Inoculum ***None***

Image: **cryo-milled sample** ***Timepoint:*** **time - 0**

Introduction

The test substance was submitted for testing according to ISO *14851 Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer*. ISO *14851* evaluates the degradation of a material by measuring the amount of oxygen consumed during the course of testing. The test substance was evaluated directly by weighing the appropriate amount into the test system with no solvents or dispersants. The test system consisted of a mineral medium inoculated with microorganisms derived from surface water in Illinois. Measurements for oxygen consumption were recorded daily for 90 days.

Substance Appearance

The test substance was provided in a form of two clear glass containers containing white/off-white powder, and each container was labeled with the ID of the test substance.

Methods

Project - Images

Project images are provided for the submitted test samples. Images are taken for the samples as received to provide a reference for the materials submitted for testing. The provided images may or may not indicate other aspects of the sample condition but no analysis or inspection of the sample is conducted unless otherwise specifically noted in the project report.

ISO 14851 - Standard for Determination of Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium - Method by Measuring the Oxygen Demand in a Closed Respirometer

Biodegradation testing is conducted according to the ISO 14851 method. Test substance is added to the sample jars directly by weight and with no additional solvent or dispersants. ISO 14851 biodegradation testing will measure the consumption of oxygen by respirometry of the microorganisms as they consume the available carbon provided by the control or test substance. At the end of testing, as determined by the test time limit or a plateau in the amount of oxygen consumed, the percent of degradation is then determined by taking the difference between the maximum oxidative capacity of the material added to the test system (control or test substance) and the final amount of oxygen utilized as measured in the test. The percent of this value represents the percent Theoretical Oxygen Demand (% ThOD). The oxidative capacity of the test substance is determined by conducting a chemical oxygen demand (COD) on the test material using acid oxidation.

A aerobic bacterial inoculum was derived from a sample acquired from a Chicagoland Waste Water Treatment Facility. The microorganism concentration is determined and recorded. An equilibration phase for the microorganism inoculum is conducted by the aging of the inoculum for approximately 7 days before introducing the test substance. Following the equilibration phase, the test substance and control material are added to each test system, and the oxygen concentration measurements are conducted.

Project List ***(notes)***

1	Hydropol 30164P
2	Hydropol 33104P
3	Negative Control
4	Reference Material - Aniline

Procedures

Inoculum

Inoculum was propagated in the mineral medium with the addition of tryptic soy broth prior to testing. On the day of testing, the concentration of microorganisms was establish and the inoculum was diluted to the final concentration of 1E5 CFU/ml.

Establishing of Chemical Oxygen Demand

The test substances were used to perform a COD analysis. The test substance was suspended in DDI water and the prepared solution was mixed by vortexing and used for COD, by addition of 2 ml of the test substance preparation to 3 ml of acid reagent and refluxed at 150 C. After 2 hours, samples were cooled to room temperature and the absorbance at 620 nm was measured and the COD was determined by comparison to a standard cuve.

Reference Substance

Name: Aniline HCl

CAS: 142-04-1

Standards

pH 4
pH 10
pH 7

Name: Dextrose
CAS: 50-99-7

Name: Potassium phthalate monobasic
CAS: 877-24-7

Name: Sodium Carbonate
CAS: 497-19-8

Name: Sodium Bicarbonate
CAS: 4144-55-8

Salt Used in Preparation of Mineral Medium

Magnesium sulfate heptahydrate

Calcium chloride dihydrate
Iron(III) chloride
Potassium phosphate dibasic trihydrate
Sodium phosphate dibasic dihydrate trihydrate
Potassium phosphate monobasic
Ammonium chloride

Chemical Oxygen Demand Reagents

HACH KIT Cat #: 2565115, Lot # A0066, Exp: March 2024

Reagents

Tryptic Soy Agar
Tryptic Soy Broth
Laboratory RO water, distilled
OECD 301 medium
Phosphate buffered saline (PBS)

Equipment List (ID #)

Laboratory and analytical balances
Laboratory Glassware
Laboratory Pipettes
Oxygen Respirometer
Thermometers
pH probe
Spiral plater
Stir plate
Laboratory DDI water
Carbon Analyzer
Spectrophotometer
COD reactor

Test Organisms (by Method) ***(Inventory ID / lot #)***

ISO 14851 - Standard for Determination of Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium - Method by Measuring the Oxygen Demand in a Closed Respirometer

municipal SKOKIE sludge isolate

Sampling Preparation

The test sample were provided as small pellets which were cryo-milled to create a fine heterogenous powder. The test substance was frozen in a 25mm ball mill with to -140C, and then milled to 1 min, and refrozen and milled for a total of 5x 1minute cycles. The powder was then used direction for analysis and testing.

Sampling Procedure

Approximatly daily oxygen meaurements were performed for the duration of testing.

Calculations

Variable Slope Sigmoidal Curve Fit (Graphpad):

Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))

EC50 is the concentration of agonist that gives a response halfway between Bottom and Top. This is not the same as the response at Y=50.

HillSlope describes the steepness of the family of curves. It is derived from the curve fit analysis for the dataset ad is a commonly used constant for first-order biological kinetics.

Top and **Bottom** are plateaus in the units of the Y-axis, (top = maximum %ThCO2, bottom = net CO2 production at time 0).

Statistical Methods

Replicate data were averaged by determination of the mean value (arithmetic average) for a selected measurement in the following manner:
A= S/N

A = average (or arithmetic mean)
S = the sum of the numbers in the set of interest (e.g., the sum of the numbers being averaged)
N = the number of terms (e.g., the number of items or numbers being averaged)

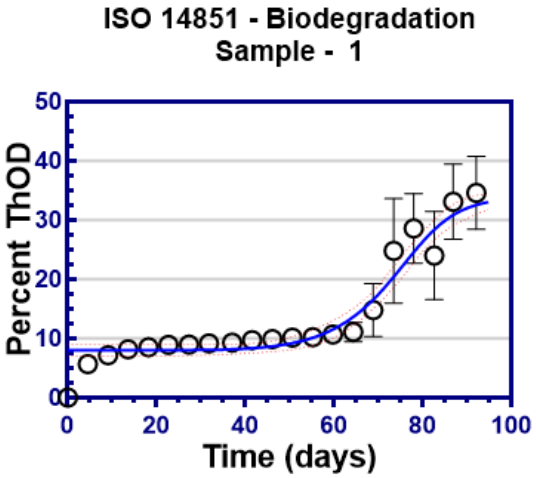
Standard deviation is a measurement of the variation around the Mean value. Built-in functions for Standard Deviation were performed within the analytic software used for calculating these values.

* This report is governed by and incorporates by reference the conditions of testing as posted on the date of issuance and is intended for the identified Project Owners exclusive use. Any copying or replication of this report to or for any other person or entity, or use of our company name or Service Mark is permitted only with our prior written consent. All images supplied as part of the report are provided as test result edification only and are the sole property of Situ Biosciences LLC and are copyright protected. Any exemption to the copyright of the report or images provided will be explicitly noted in this report.

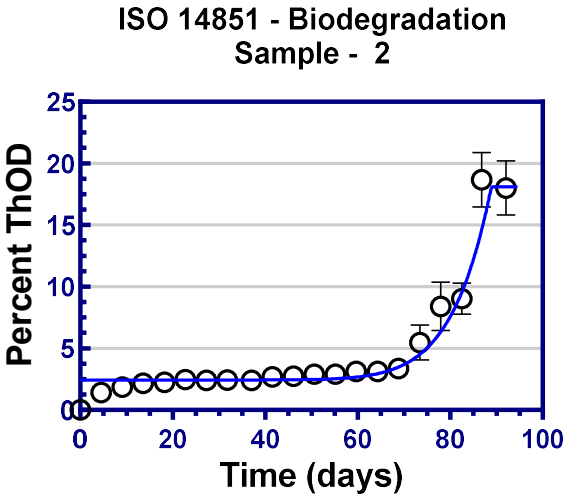
This report sets forth our findings solely with respect to test samples identified herein. The results set forth in this report are not indicative or representative of the quality or characteristics of the lot from which a test sample was taken or any similar identical product unless specifically and expressly noted. Our report includes all tests requested and the results thereof based upon the information provided. Written notification within 60 days from the date of issuance of this report is required to address any material error or omission caused by the handling of the samples. Any such notification shall specifically address the issues related to the test samples supplied and testing conducted as provided in this report. A failure to raise such an issue within the prescribed time shall constitute the unqualified acceptance of the completeness of this report, the testing conducted, and the correctness of the report contents.

d.p. satchell, Ph.D.

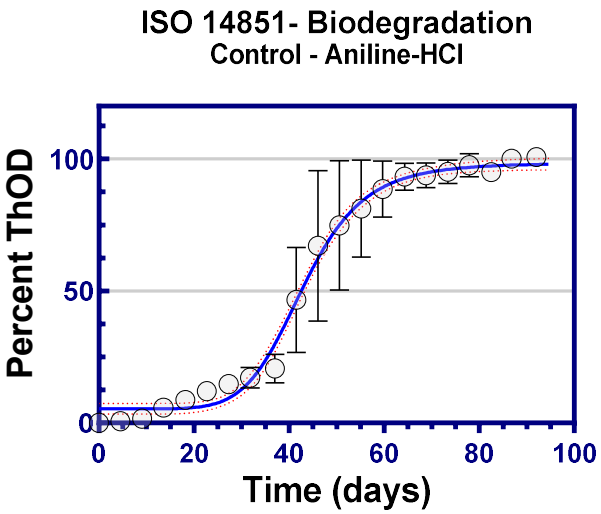
Technical Manager



Time (days)	Percent ThO2			Time (days)	Percent ThO2		
0	0	0	0	46.1	11.5	7.6	10.6
0.9	3.1	2.4	2.4	47	11.6	7.6	10.7
1.8	4.2	3	3.1	47.9	11.5	7.5	10.7
2.7	5.3	3.9	3.9	48.8	11.5	7.5	10.8
3.6	6.2	4.5	4.5	49.7	11.5	7.5	10.9
4.5	7.2	4.9	4.9	50.6	11.7	7.6	11.1
5.5	8.3	5.2	5.5	51.5	11.7	7.6	11.2
6.4	8.6	5.4	5.8	52.5	11.9	7.7	11.5
7.3	8.6	5.5	5.9	53.4	11.9	7.7	11.5
8.2	8.8	5.7	6.2	54.3	11.7	7.5	11.3
9.1	9.1	5.9	6.5	55.2	11.8	7.5	11.4
10	9.5	6.2	6.9	56.1	12.1	7.8	11.7
10.9	9.6	6.2	6.9	57	12.1	7.8	11.8
11.8	9.9	6.5	7.2	57.9	12.5	8	12.1
12.7	10	6.6	7.4	58.8	12.2	7.8	12
13.6	10.2	6.7	7.6	59.7	12.2	7.8	12
14.5	10.3	6.9	7.8	60.6	12.3	7.8	12.2
15.5	10.6	7.1	8	61.5	12.4	8	12.3
16.4	10.6	7.1	8.1	62.5	12.6	8.1	12.6
17.3	10.5	7	8	63.4	12.5	7.9	12.5
18.2	10.5	7	8	64.3	13	7.9	12.4
19.1	10.7	7.2	8.2	65.2	18.8	7.9	12.4
20	10.8	7.3	8.4	66.1	20.5	8.3	12.7
20.9	10.7	7.2	8.4	67	21.4	8.3	12.8
21.8	10.8	7.2	8.5	67.9	22.9	8.3	12.7
22.7	10.9	7.3	8.6	68.8	23.4	8.3	12.7
23.6	10.9	7.3	8.6	69.7	24.1	8.3	12.7
24.5	10.8	7.2	8.6	70.6	23.8	10.5	16.8
25.5	11.2	7.5	9	71.6	28.2	13.9	17
26.4	11	7.2	8.9	72.5	36	14.3	17.2
27.3	10.9	7.2	8.8	73.4	42.4	14.7	17.3
28.2	10.9	7.2	8.8	74.3	42.4	14.7	17.3
29.1	10.9	7.2	8.9	75.2	42.4	15.4	17.3
30	11	7.2	9.1	76.1	42.4	19.5	17.3
30.9	10.9	7.2	9.1	77	39.8	17	14.7
31.8	11	7.3	9.3	77.9	39.8	26.1	19.9
32.7	11	7.3	9.3	78.8	35.4	21.6	15.5
33.6	11.1	7.4	9.4	79.7	35.2	21.8	18.5
34.6	11	7.3	9.4	80.6	42.2	21.8	18.5
36.1	11.1	7.4	9.5	81.6	40.1	18.2	13.9
37	11.1	7.4	9.4	82.5	39	17.1	16
37.9	11.2	7.5	9.7	83.4	37.6	22.7	14.6
38.8	11.2	7.5	9.7	84.1	37.6	22.7	14.6
39.7	11.3	7.6	9.9	85	37.6	22.7	14.6
40.6	11.2	7.5	10	85.9	40.4	24.4	18.4
41.5	11.4	7.6	10.2	86.8	44.6	31.9	22.8
42.5	11.6	7.8	10.5	87.7	44.6	34.4	22.8
43.4	11.3	7.5	10.3	88.6	43.9	33.8	24.3
44.3	11.3	7.4	10.3	89.5	43.9	33.8	24.3
45.2	11.3	7.4	10.3	91.1	43.9	33.8	24.3
				92	45.7	33.8	24.3
				92.9	47.1	34	24.5
				93.8	47.8	34.1	24.6
				94.8	48.2	34	24.6



Time (days)	Percent ThO2			Time (days)	Percent ThO2		
0	0	0	0	46.1	2.8	3.1	2.3
0.9	0.7	0.6	0.5	47	2.9	3.2	2.4
1.8	1	1	1	47.9	2.8	3.2	2.3
2.7	1.3	1.1	1.1	48.8	2.8	3.2	2.3
3.6	1.5	1.4	1.3	49.7	2.8	3.2	2.3
4.5	1.5	1.4	1.3	50.6	2.9	3.4	2.4
5.5	1.9	1.7	1.6	51.5	2.9	3.4	2.5
6.4	2	1.9	1.7	52.5	3.1	3.6	2.6
7.3	1.8	1.7	1.6	53.4	3	3.5	2.5
8.2	1.9	1.8	1.6	54.3	2.9	3.4	2.4
9.1	2	1.9	1.7	55.2	2.9	3.4	2.4
10	2.2	2.1	1.9	56.1	3.1	3.6	2.6
10.9	2.1	2	1.8	57	3.1	3.7	2.6
11.8	2.2	2.1	1.9	57.9	3.3	3.9	2.8
12.7	2.2	2.2	1.9	58.8	3.1	3.7	2.6
13.6	2.2	2.3	2	59.7	3.1	3.7	2.6
14.5	2.3	2.3	2.1	60.6	3.1	3.7	2.6
15.5	2.4	2.5	2.2	61.5	3.1	3.9	2.6
16.4	2.3	2.5	2.2	62.5	3.3	4	2.7
17.3	2.2	2.4	2.1	63.4	3.1	3.9	2.6
18.2	2.2	2.4	2.1	64.3	3.1	3.8	2.5
19.1	2.4	2.5	2.3	65.2	3.1	3.8	2.5
20	2.5	2.7	2.5	66.1	3.3	4	2.7
20.9	2.4	2.5	2.3	67	3.4	4.2	2.9
21.8	2.5	2.6	2.4	67.9	3.3	4.1	2.7
22.7	2.5	2.6	2.3	68.8	3.3	4.1	2.7
23.6	2.5	2.6	2.3	69.7	3.3	4.3	2.7
24.5	2.4	2.5	2.2	70.6	7.7	4.9	2.7
25.5	2.8	2.8	2.5	71.6	7.8	5.1	2.8
26.4	2.6	2.6	2.3	72.5	8	5.2	2.9
27.3	2.5	2.5	2.2	73.4	8	5.3	3.1
28.2	2.5	2.5	2.2	74.3	8	5.3	3.1
29.1	2.5	2.5	2.2	75.2	8	5.3	3.1
30	2.6	2.6	2.2	76.1	8	5.3	3
30.9	2.5	2.6	2.2	77	8.4	5.7	7.3
31.8	2.5	2.6	2.2	77.9	12.2	5.7	7.3
32.7	2.5	2.6	2.2	78.8	7.8	5.5	6.8
33.6	2.6	2.7	2.2	79.7	10.4	7.6	9.1
34.6	2.5	2.6	2.2	80.6	10.4	7.6	9.1
36.1	2.5	2.7	2.1	81.6	6.7	4.2	5.7
37	2.5	2.6	2.1	82.5	11.5	7.4	8.2
37.9	2.6	2.8	2.2	83.4	10.1	6	6.8
38.8	2.6	2.8	2.2	84.1	10.1	6	6.8
39.7	2.8	3	2.3	85	10	5.9	6.8
40.6	2.7	2.9	2.3	85.9	17	9.4	11.6
41.5	2.8	3	2.3	86.8	22.8	15.2	18
42.5	3	3.2	2.5	87.7	22.8	15.2	18
43.4	2.7	3	2.3	88.6	22.1	14.6	17.4
44.3	2.7	2.9	2.2	89.5	22.1	14.6	17.4
45.2	2.7	2.9	2.2	91.1	22.1	14.6	17.4
				92	22.1	14.6	17.3
				92.9	22.3	14.7	17.5
				93.8	22.4	14.8	17.5
				94.8	22.3	14.8	17.5



Time (days)	Percent ThO2			Time (days)	Percent ThO2		
0	0	0	0	46.1	34.2	83.6	83.6
0.9	0.2	0.2	0.2	47	36.6	85.1	85.1
1.8	0.5	0.4	0.4	47.9	38.9	86.3	86.3
2.7	0.6	0.5	0.5	48.8	41.3	87.3	87.3
3.6	0.7	0.6	0.6	49.7	43.8	88.2	88.2
4.5	0.7	0.7	0.7	50.6	46.5	89	89
5.5	0.8	0.8	0.8	51.5	49.2	89.7	89.7
6.4	1.1	1	1	52.5	51.9	90.5	90.5
7.3	1.1	1	1	53.4	54.6	91.1	91.1
8.2	1.3	1.2	1.2	54.3	57.3	91.5	91.5
9.1	1.7	1.6	1.6	55.2	60	91.9	91.9
10	2.2	2	2	56.1	63.1	92.6	92.6
10.9	2.7	2.6	2.6	57	66.3	93.2	93.2
11.8	3.7	3.7	3.7	57.9	69.6	93.8	93.8
12.7	5.3	4.5	4.5	58.8	72.9	94.3	94.3
13.6	7.3	5.1	5.1	59.7	76.4	94.8	94.8
14.5	8.6	5.6	5.6	60.6	80	95.2	95.2
15.5	9.2	6.3	6.3	61.5	83.6	95.7	95.7
16.4	9.6	7	7	62.5	86.2	96.1	96.1
17.3	9.8	7.6	7.6	63.4	86.9	96.3	96.3
18.2	9.9	8.1	8.1	64.3	87.4	96.3	96.3
19.1	10.2	9	9	65.2	87.7	96.4	96.4
20	10.6	10	10	66.1	88.2	96.6	96.6
20.9	10.8	10.8	10.8	67	88.4	96.7	96.7
21.8	11	11.7	11.7	67.9	88.2	96.6	96.6
22.7	11.2	12.5	12.5	68.8	88.4	96.6	96.6
23.6	11.3	13.2	13.2	69.7	88.7	96.7	96.7
24.5	11.5	14	14	70.6	89	96.9	96.9
25.5	11.8	14.9	14.9	71.6	89.4	97.2	97.2
26.4	11.9	15.6	15.6	72.5	89.7	97.4	97.4
27.3	12	16.2	16.2	73.4	90	97.7	97.7
28.2	12	16.7	16.7	74.3	90	97.7	97.7
29.1	12.1	17.3	17.3	75.2	90	97.7	97.7
30	12.3	18.1	18.1	76.1	90	97.7	97.7
30.9	12.4	18.7	18.7	77	92.6	100.2	100.2
31.8	12.6	19.4	19.4	77.9	92.6	100.2	100.2
32.7	12.7	20.1	20.1	78.8	92.7	98.5	98.5
33.6	13	20.8	20.8	79.7	93.7	98.4	98.4
34.6	13.3	21.6	21.6	80.6	93.7	98.4	98.4
36.1	13.6	22.1	22.1	81.6	92.4	95.2	95.2
37	14.4	23.8	23.8	82.5	94.9	95.1	95.1
37.9	15.8	27.9	27.9	83.4	94	94.2	94.2
38.8	17.3	35.1	35.1	84.1	94	94.2	94.2
39.7	19.3	44	44	85	94	94.2	94.2
40.6	21.3	51	51	85.9	97.1	96.7	96.7
41.5	23.6	58.1	58.1	86.8	100.6	99.9	99.9
42.5	25.9	66.4	66.4	87.7	100.6	99.9	99.9
43.4	27.9	75.9	75.9	88.6	100.2	101.1	101.1
44.3	30	79.7	79.7	89.5	100.2	101.1	101.1
45.2	31.9	81.8	81.8	91.1	100.2	101.1	101.1
				92	100.2	101.1	101.1
				92.9	100.4	101.3	101.3
				93.8	100.5	101.5	101.5
				94.8	100.5	101.6	101.6