

***Study Title***

**H-28397: ACTIVATED SLUDGE RESPIRATION INHIBITION TEST  
(OECD 209)**

***Test Guideline***

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

***Authors***

Robert F. Vavala and William R. Berti, Ph.D.

***Study Completion Date***

05-September-2008

***Revision No. 1 Completion Date***

21-October-2008

***Test Facility***

DuPont Haskell Global Centers for Health & Environmental Sciences  
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***Submitter***

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U.S.A.

***Work Request/Service Code***

17568 / 1674

***DuPont Report Number***

25938

**PAGE RESERVED**

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report, with the exception of the items listed below was conducted in compliance with the following GLP Standards:

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792

which are consistent with:

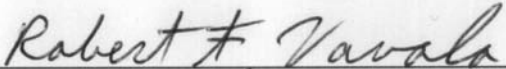
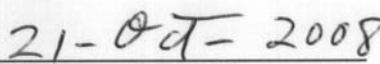
The OECD Principles on Good Laboratory Practice (as revised 1997),  
ENV/MC/CHEM98(17), OECD, Paris, 1998

MAFF Japan Good Laboratory Practice Standards (11 Nousan Number 6283).

The following items are exceptions to the GLP Standards. These items do not impact the validity of the study.

The reference substance was not characterized under GLP. The test substance was provided by the sponsor. The reference substance is a commercially available material provided by a commercial supplier. The Certificates of Analysis were provided by the sponsor and supplier and the accuracy of the data is considered sufficient for the purposes of this study.

### *Study Director*

	
Robert F. Vavala	Date
Staff Scientist	
DuPont Haskell Global Centers for Health & Environmental Sciences	

### *Sponsor/Submitter*

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Date

## QUALITY ASSURANCE STATEMENT

### *Study Number*

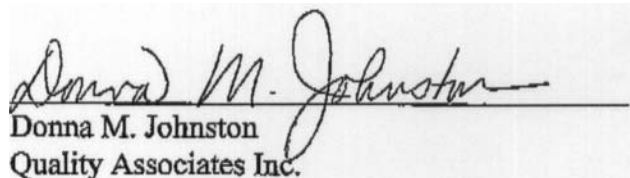
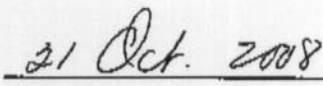
25938

### *Study Title*

H-28397: Activated Sludge Respiration Inhibition Test (OECD 209).

The conduct of this study was subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below:

<b>Study Phase Inspected</b>	<b>Inspection/Audit Dates</b>	<b>Dates Findings Reported to Study Director</b>	<b>Dates Findings Reported to Management</b>
Protocol	15-April-2008	15-April-2008	21-April-2008
Study setup up and dosing	24-April-2008	16-June-2008	16-June-2008
Study records and final report	04-August-2008	08-August-2008	05-Sept.-2008
Report Revision No. 1	16-October-2008	16-October-2008	16-October-2008

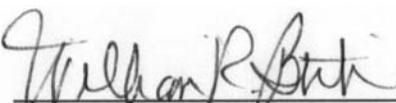
 Donna M. Johnston Quality Associates Inc.	 Date
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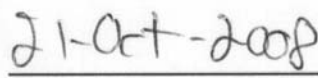
## **CERTIFICATION OF AUTHENTICITY**

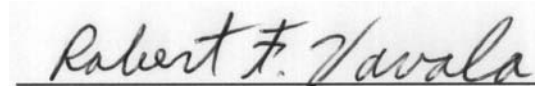
### **H-28397: ASSESSMENT OF BIODEGRADABILITY BY THE ACTIVATED SLUDGE RESPIRATION INHIBITION TEST (OECD 209)**

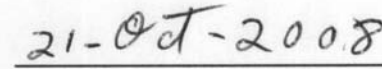
We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

***Report by:***

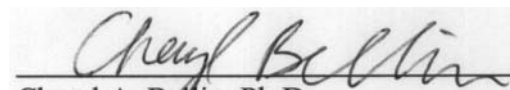
  
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William R. Berti, Ph.D.  
Research Associate

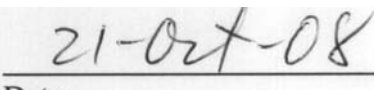
  
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Date

  
\_\_\_\_\_  
Robert F. Vavala  
Staff Scientist

  
\_\_\_\_\_  
Date

***Approved by:***

  
\_\_\_\_\_  
Cheryl A. Bellin, Ph.D.  
Research Manager

  
\_\_\_\_\_  
Date

***Study Initiation Date:***

23-April-2008

***Date Study Completed:***

05-September-2008

***Revision No. 1 Completion Date:***

21-October-2008

***Submitter:***

James R. Hoover  
DuPont Co.  
Wilmington, Delaware 19880 U.S.A.

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## **H-28397: ACTIVATED SLUDGE RESPIRATION INHIBITION TEST (OECD 209)**

### *Authors*

Robert F. Vavala and William R. Berti, Ph.D.

## **0.0 REASONS FOR REVISION**

- The WR number appearing on the title page was corrected.
- The notebook number appearing on the first page was moved to page 8.

## **1.0 SUMMARY**

### *Test System:*

H-28397 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 10, 32, 100, 320, and 1000 mg L<sup>-1</sup> nominal concentrations. For the reference substance 3,5-dichlorophenol, activated sludge was exposed at 3.2, 10, and 32 mg L<sup>-1</sup> nominal concentrations. After a three hour incubation period, the inhibition of the respiration rate of the activated sludge was determined in comparison to a test solution without any test or reference substance.

### *Findings:*

Under the conditions of the test, there was no significant activated sludge respiration inhibition (inhibition less than 15%) at concentrations of the test substance H-28397 as high as 1000 mg L<sup>-1</sup> (1000 ppm) compared to the positive controls to which the test substance was not added.

The Effective Concentration of the reference substance 3-5, dichlorophenol at which 50% inhibition occurred (EC<sub>50</sub>) was approximately 10 mg L<sup>-1</sup>.

The difference between the respiration rates of the two positive controls measured at the start and end of the test was less than 10%.

### *Conclusions:*

The Effective Concentrations of the test substance at which 20, 50, and 80% inhibition occurred (EC<sub>20</sub>, EC<sub>50</sub>, EC<sub>80</sub>, respectively) could not be determined because there was no inhibition at the highest test concentration of 1000 mg L<sup>-1</sup>.

The test is valid.

## 2.0 GENERAL STUDY INFORMATION

### *Study Objectives*

The aim of this study was the determination of the acute toxic behaviour of H-28397 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, H-28397, on microorganisms from municipal sewage sludge, using a microbial inoculum and a artificial sewage feed and measuring the respiration rate of the test system after a three (3) hour time period under controlled laboratory conditions.
- Suitable non-inhibitory concentrations of the test substance to be used in biodegradability tests

### *Test System Justification*

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

### *Study Personnel*

Management:	Cheryl A. Bellin, Ph.D.
Study Director:	Robert F. Vavala DuPont Haskell Global Centers for Health & Environmental Sciences Central Research & Development, Glasgow Building 300 PO Box 6300 Newark, DE 19714-6101 USA
Technical Personnel:	William R. Berti, Ph.D. Andrew Grosik
Notebook Number	E105558-DW

### *Study Execution Dates*

Experimental Start Date:	24-April-2008
Experimental Completion Date:	08-May-2008
Study Completion Date:	05-September-2008
Revision No. 1 Completion Date	21-October-2008



## 3.0 MATERIALS AND METHODS

### 3.1 *Test Guidelines*

The purpose of the procedure is to provide a screening method to identify substances that may adversely affect aerobic microorganisms from municipal sewage treatment plants. The procedure also can 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 three hours 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:	H-28397
Synonyms/Codes:	HFPO Dimer Acid Ammonium Salt
CAS Name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
CAS Registry Number:	62037-80-3
Purity:	88%
Other components:	
Water	13.3%
Perfluorooctanoic Acid	3.4 ppm

##### 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:	Alfa Aesar L02052
Lot Number:	A10104261
EMSE Sample No.	CRD 14,758
Purity:	99.3%

**3.2.1.3**    *Test Vehicle*

The test substance was dissolved in a stock solution of Barnstead Diamond™ water at 10,000 mg L<sup>-1</sup>. The concentration of the test substance in the stock solution was determined to be 9370 mg H-28397 L<sup>-1</sup>.

**3.2.1.4**    *Application Information*

The test substance was added to the test vessels at the following nominal concentrations: 1000, 320, 100, 32 and 10 mg L<sup>-1</sup>.

**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 kept aerated and fed with synthetic sewage feed. The amount of sludge to use as inoculum is determined by measuring its respiration rate at 50, 100, and 200 mL of sludge after diluting with 16 mL of synthetic sewage feed and sufficient dechlorinated water for a final volume of 500mL. The respiration rate will be measured after a minimum mixing time of 30 minutes. This pre-experiment to determine the amount of sludge to use as inoculum will not be performed in compliance with the GLP-Regulations. The raw data, however, will be included in the study records and will be archived under the project number of the study.

**3.2.3**        *Physical System*

**3.2.3.1**    *Test Units*

All test vessels are 1000 mL glass flasks and contain a final volume of 500 mL. Test solutions were transferred to 300-mL Biological Oxygen Demand (BOD) glass bottles for Dissolved Oxygen (DO) determinations.

**3.2.3.2**    *Test Conditions*

Test solutions were aerated with compressed air at a flow rate of approximately 0.1 to 0.5 liter per minute at room temperature.

**3.3**            *Test Conduct*

Prior to the start of the test, all components were added to the test vessels, less the volume of the inoculum. This volume is determined by a pre-test of the inoculum to find a concentration that gives an acceptable respiration rate. The test was initiated with the first positive control by adding the microbial inoculum and aeration started. After approximately 15 minutes the inoculum was added to the first reference substance. The procedure was repeated at approximately 15-minute intervals with the reference substance and then the test substance to give a series of vessels containing different concentrations of the reference and test substance. A negative control (synthetic sewage feed and test substance at the highest concentration, but without microbial inoculum) test was also evaluated. The final flask was a second positive control, prepared exactly as the first.

The test was conducted in the following order so that the reference and test substances were bracketed by the two controls:

- first positive control
- reference substance at 3 concentrations
- test substance at 5 concentrations
- negative (abiotic) control at highest concentration of test substance
- second positive control

### **3.4 *Parameters Observed***

#### **3.4.1 *Analysis of Test Systems***

For the measurement of the respiration rate a well-mixed sample of each treatment was transferred into a BOD bottle after 3 hours incubation time, and was not further aerated. The oxygen concentration was measured with an oxygen electrode and recorded over a period of about 10 minutes or until the dissolved oxygen (DO) concentration fell below about 1 mg DO L<sup>-1</sup>. During measurement, the samples were continuously stirred with the built-in stirrer. The rate of oxygen consumption (in mg O<sub>2</sub> L<sup>-1</sup> h<sup>-1</sup>) was determined from the most linear part of the respiration curve.

Test sample temperature was measured concurrently with the DO measurements and recorded.

#### **3.4.2 *Analysis of the Test and Reference Substance Stock Solutions***

Concentrations of the test and reference substance stock solutions were estimated by determining the total dissolved organic carbon in one liter of water to which approximately 500 mg of test or reference substance had been added and mixed. Samples were analyzed for total carbon content via a Shimadzu TOC-V analyzer with an autosampler attachment. This system is based on the combustion/non-dispersive infrared gas analysis method widely employed for TOC measurement. Carbon dioxide-free carrier gas flows to the combustion tube, which is filled with an oxidation catalyst and heated to 680 °C. The total carbon (TC) of a sample is burned in the combustion tube to form carbon dioxide. The carrier gas, containing the carbon dioxide and other combustion products, flows from the combustion tube to an electronic dehumidifier, where it is cooled and dehydrated. The gas then passes through a halogen scrubber before going through the non-dispersive infrared (NDIR) gas analyzer, where the carbon dioxide is detected. The analog detection signal of the NDIR forms a peak, which is proportional to the TC concentration of the sample. A calibration curve equation that mathematically expresses the relationship between peak area and TC concentration is generated by analyzing various concentrations of a TC standard solution. The TC concentration in a sample can be determined by analyzing the sample to obtain the peak area and then using the peak area in the calibration curve equation.

Concentration was calculated using the following equation:

$$C = \frac{TOC * MW_s}{MW_c * CA}$$

where:

C =	Concentration, mg L <sup>-1</sup>
TOC =	Total Dissolved Organic Carbon, mg L <sup>-1</sup>
MW <sub>s</sub> =	Molecular Weight of the substance, mg mmole <sup>-1</sup>
MW <sub>c</sub> =	Molecular Weight of Carbon, 12.01 mg mmole <sup>-1</sup>
CA =	Number of Carbon atoms in test substance

### 3.5 *Result Analysis*

#### 3.5.1 *Respiration Rate*

The respiration rate was calculated from the output as mg O<sub>2</sub> L<sup>-1</sup> h<sup>-1</sup>. This was done by graphing mg O<sub>2</sub> L<sup>-1</sup> 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 was 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<sub>2</sub> L<sup>-1</sup> 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 will be determined by comparing the respiration rates at each concentration of either test or reference substance to the respiration rates in the controls. The results will be 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

$R_s$  = oxygen consumption rate at tested concentration of test or reference substance

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

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

The 3-hour  $EC_{50}$  (Effective Concentration of the substance giving a calculated or interpolated inhibition of oxygen consumption of 50% compared with a blank control). 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) are within 15 percent of each other:
- The  $EC_{50}$  (3 hours) of 3,5-dichlorophenol is in the accepted range of 5 to 30  $\text{mg} \cdot \text{L}^{-1}$ , 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.

## 4.0 RESULTS AND DISCUSSION

Under the conditions of the test, there was no significant activated sludge respiration inhibition (inhibition less than 15%) at concentrations of the test substance H-28397 as high as 1000  $\text{mg} \cdot \text{L}^{-1}$  (1000 ppm) compared to the positive controls to which the test substance was not added.

The Effective Concentration of the reference substance 3-5, dichlorophenol at which 50% inhibition occurred ( $EC_{50}$ ) was approximately 10  $\text{mg} \cdot \text{L}^{-1}$ .

The difference between the respiration rates of the two positive controls measured at the start and end of the test was less than 10%.

## **5.0 CONCLUSIONS**

The Effective Concentrations of the test substance at which 20, 50, and 80% inhibition occurred (EC<sub>20</sub>, EC<sub>50</sub>, EC<sub>80</sub>, respectively) could not be determined because there was no inhibition at the highest test concentration of 1000 mg L<sup>-1</sup>.

The test is valid.

## **6.0 RETENTION OF RECORDS**

Study documents and materials will be archived at DuPont Haskell Global Center for Health and Environmental Sciences, Glasgow, Delaware, 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.

## **7.0 RETENTION OF TEST SUBSTANCE**

After issuance of the final report, the remaining test substance will be stored at the DuPont Haskell Global Center Lab, Glasgow, Delaware until its expiration date. If the sponsor wishes that a portion is returned, a sample of the test substance will be retained at the Laboratory.

## **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 H-28397 AND REFERENCE SUBSTANCE 3, 5-DICHLOROPHENOL ON THE O<sub>2</sub> CONSUMPTION OF ACTIVATED SLUDGE.**

Treatment	Nominal Concentration	Concentration based on Stock Solution Determination	O <sub>2</sub> Concentration†		O <sub>2</sub> Consumption by regression	Inhibition‡
			Start	End		
	mg/L	mg/L	mg O <sub>2</sub> /L		mg O <sub>2</sub> /L/h	%
Positive control 1	0	0	3.18	0.06	25.7	NA‡
Positive control 2	0	0	5.96	2.16	23.4	NA
Mean					24.5	NA
Difference, %					9.5	NA
3,5- Dichlorophenol(32ml)	32	32	7.04	6.35	4.5	81.8
3,5- Dichlorophenol(10ml)	10	10	6.53	4.69	11.2	54.3
3,5- Dichlorophenol(3.2ml)	3.2	3.2	5.03	1.43	22.5	8.3
TEST#1	1000	1000	4.96	1.10	23.1	5.8
TEST#2	320	320	0.57	0.05	27.6	-12.5
TEST#3	100	100	4.6	0.05	26.4	-7.6
TEST#4	32	32	3.53	0.06	23.8	3.0
TEST#5	10	10	2.73	0.05	26.5	-8.1
Abiotic Control	1000	1000	8.35	8.6	0.68	97.2

†The readings of the Dissolved O<sub>2</sub> meter at start and end of data collection period after 3-h of aeration.

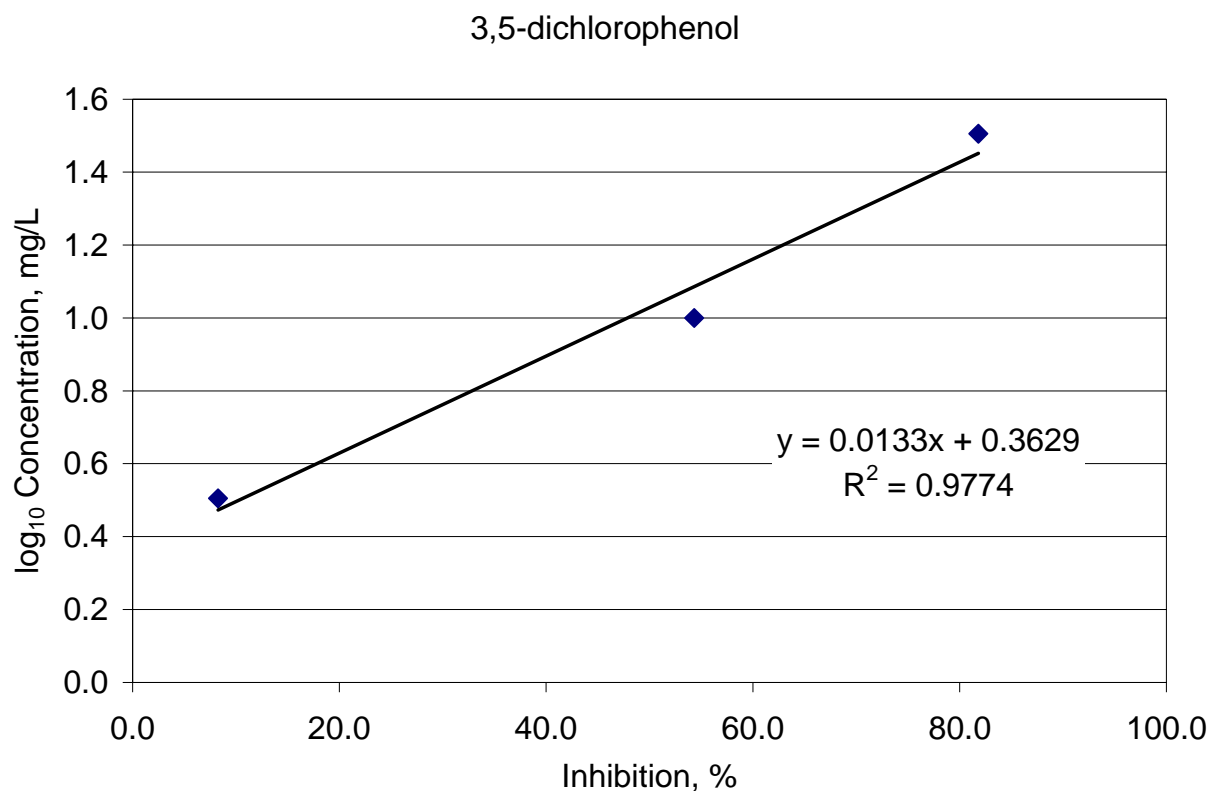
‡NA = Not Applicable.

**TABLE 2:**  
**TOTAL ORGANIC CARBON AND CALCULATED CONCENTRATIONS OF THE TEST AND REFERENCE SUBSTANCES**  
**STOCK SOLUTIONS.**

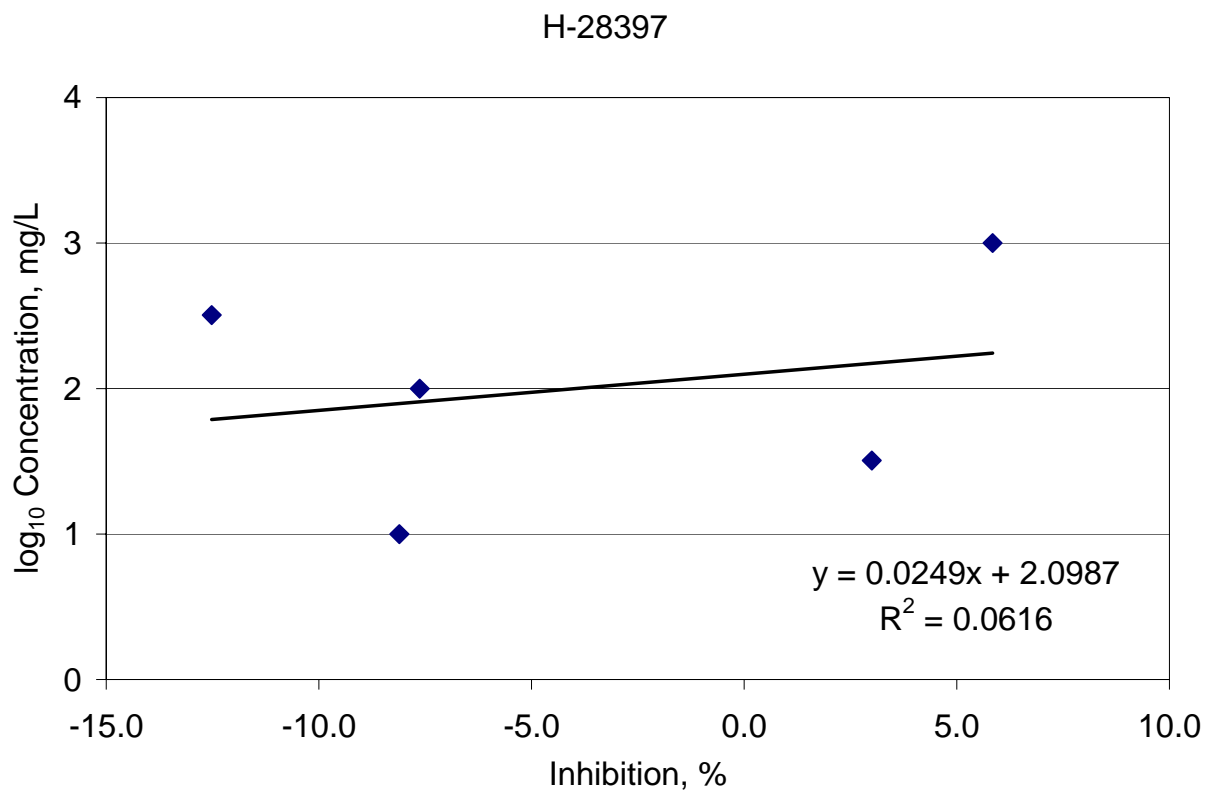
	Nominal Concentration	Carbon Molecules, CA	Molecular Weight, MWs	Total Organic Carbon, TOC	Stock Solution Concentration, C
	<i>mg /L</i>	<i>number</i>	<i>g/mole</i>	<i>mg TOC /L</i>	<i>mg /L</i>
H-28397	10000	6	347.1	1229	9370
3,5-dichlorophenol	500	6	163	279.5	632



**FIGURE 1:**  
**INFLUENCE OF THE REFERENCE SUBSTANCE 3,5-DICHLOROPHENOL ON THE**  
**RESPIRATION RATE OF AEROBIC WASTEWATER MICROORGANISMS AFTER 3 HOURS**  
**OF EXPOSURE.**



**FIGURE 2:**  
**INFLUENCE OF THE TEST SUBSTANCE H-28397 ON THE RESPIRATION RATE OF**  
**AEROBIC WASTEWATER MICROORGANISMS AFTER 3 HOURS OF EXPOSURE**



**APPENDIX A:**  
**CERTIFICATE OF ANALYSIS OF H-28397.**



E. I. du Pont de Nemours and Company  
Wilmington, DE 19898  
USA

**CERTIFICATE OF ANALYSIS**

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28397
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	88%
Other Components	Water – 13.3% (non-GLP) Perfluorooctanoic acid – 3.4 ppm
Date of Analysis	November 16, 2007
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-24127
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Experimental Station Wilmington, Delaware USA

Approver:

A handwritten signature in black ink, appearing to read "Peter A. Bloxham", written over a horizontal line.

Peter A. Bloxham, Ph.D.  
Senior Research Chemist

19-Nov-2007  
Date

**APPENDIX B:**

**DATA TABLES AND CALCULATIONS FOR POSITIVE CONTROL, REFERENCE AND TEST SUBSTANCES, AND NEGATIVE CONTROL SAMPLES.**

*Positive control 1*

---

<b>Readin g Number</b>	<b>Time</b>	<b>DO Reading</b>
	<i>sec</i>	<i>mg/L</i>
1	0	3.18
2	30	2.62
3	60	2.32
4	90	2.08
5	120	1.87
6	150	1.65
7	180	1.44
8	210	1.23
9	240	1.01
10	270	0.8
11	300	0.58
12	330	0.38
13	360	0.17
14	390	0.08
15	420	0.07
16	450	0.06
17	480	0.06
18	510	0.06
19	540	0.06

---

*Positive control 2*

---

<b>Readin g Number</b>	<b>Time</b>	<b>DO Reading</b>
	<i>sec</i>	<i>mg/L</i>
1	0	5.96
2	30	5.68
3	60	5.48
4	90	5.29
5	120	5.10
6	150	4.91
7	180	4.70
8	210	4.48
9	240	4.30
10	270	4.08
11	300	3.90
12	330	3.76
13	360	3.61
14	390	3.41
15	420	3.14
16	450	2.93
17	480	2.74
18	510	2.53
19	540	2.35
20	570	2.16

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Reference substance 32:

32 mg/L 3,5-dichlorophenol

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Reading Number	Time sec	DO Reading mg/L
1	0	7.04
2	30	6.98
3	60	6.95
4	90	6.91
5	120	6.89
6	150	6.85
7	180	6.80
8	210	6.77
9	240	6.73
10	270	6.69
11	300	6.67
12	330	6.63
13	360	6.6
14	390	6.56
15	420	6.52
16	450	6.48
17	480	6.45
18	510	6.38
19	540	6.35

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Reference substance 10:

10 mg/L 3,5-dichlorophenol

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Readin g Number	Time sec	DO Reading mg/L
1	0	6.53
2	30	6.35
3	60	6.25
4	90	6.17
5	120	6.06
6	150	5.97
7	180	5.88
8	210	5.78
9	240	5.69
10	270	5.59
11	300	5.51
12	330	5.42
13	360	5.33
14	390	5.23
15	420	5.15
16	450	5.06
17	480	4.96
18	510	4.87
19	540	4.78
20	570	4.69

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Reference substance 3.2: 3.2 mg/L 3,5-  
dichlorophenol

Reading Number	Time sec	DO Reading mg/L
1	0	5.03
2	30	4.78
3	60	4.58
4	90	4.39
5	120	4.21
6	150	4.02
7	180	3.83
8	210	3.64
9	240	3.46
10	270	3.26
11	300	3.08
12	330	2.90
13	360	2.71
14	390	2.52
15	420	2.35
16	450	2.16
17	480	1.92
18	510	1.80
19	540	1.61
20	570	1.43

Test substance 1000: 1000 mg/L H-28397

Readin g Number	Time sec	DO Reading mg/L
1	0	4.96
2	30	4.51
3	60	4.31
4	90	4.11
5	120	3.92
6	150	3.74
7	180	3.56
8	210	3.38
9	240	3.17
10	270	2.99
11	300	2.81
12	330	2.62
13	360	2.43
14	390	2.23
15	420	2.06
16	450	1.69
17	480	1.71
18	510	1.52
19	540	1.33
20	570	1.10

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*Test substance 320: 320 mg/L H-28397*

<b>Readin g Number</b>	<b>Time</b>	<b>DO Reading</b>
	<i>sec</i>	<i>mg/L</i>
1	0	0.57
2	30	0.24
3	60	0.11
4	90	0.08
5	120	0.07
6	150	0.06
7	180	0.06
8	210	0.06
9	240	0.06
10	270	0.06
11	300	0.06
12	330	0.05
13	360	0.05
14	390	0.05
15	420	0.05
16	450	0.05
17	480	0.05
18	510	0.05
19	540	0.05
20	570	0.05

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*Test substance 100: 100 mg/L H-28397*

<b>Readin g Number</b>	<b>Time</b>	<b>DO Reading</b>
	<i>sec</i>	<i>mg/L</i>
1	0	4.60
2	30	0.76
3	60	0.36
4	90	0.14
5	120	0.10
6	150	0.08
7	180	0.07
8	210	0.06
9	240	0.06
10	270	0.06
11	300	0.05
12	330	0.05
13	360	0.05
14	390	0.05
15	420	0.05
16	450	0.05
17	480	0.05
18	510	0.05
19	540	0.05
20	570	0.05

Test substance 32: 32 mg/L H-28397

Readin g Number	Time sec	DO Reading mg/L
1	0	3.53
2	30	3.21
3	60	2.98
4	90	2.75
5	120	2.54
6	150	2.33
7	180	2.12
8	210	1.92
9	240	1.75
10	270	1.53
11	300	1.33
12	330	1.13
13	360	0.92
14	390	0.72
15	420	0.52
16	450	0.32
17	480	0.13
18	510	0.07
19	540	0.06
20	570	0.06

Test substance 10: 10 mg/L H-28397

Readin g Number	Time sec	DO Reading mg/L
1	0	2.73
2	30	2.47
3	60	2.21
4	90	1.91
5	120	1.70
6	150	1.46
7	180	1.21
8	210	0.97
9	240	0.70
10	270	0.47
11	300	0.24
12	330	0.09
13	360	0.07
14	390	0.06
15	420	0.06
16	450	0.06
17	480	0.06
18	510	0.06
19	540	0.05
20	570	0.05



*Negative control: Negative control with  
1000 mg H-28397 /L*

<b>Readin g Number</b>	<b>Time</b>	<b>DO Reading</b>
	<i>sec</i>	<i>mg/L</i>
1	0	8.35
2	30	8.40
3	60	8.42
4	90	8.43
5	120	8.41
6	150	8.41
7	180	8.42
8	210	8.42
9	240	8.41
10	270	8.43
11	300	8.44
12	330	8.50
13	360	8.49
14	390	8.47
15	420	8.47
16	450	8.48
17	480	8.48
18	510	8.48
19	540	8.20
20	570	8.60