ExxonMobil BIOMEDICAL SCIENCES, INC.

EMBSI 2010-104821

READY BIODEGRADABILITY: OECD 301F
Manometric Respirometry Test

Final Report

Study Number: 1057679

TEST SUBSTANCE:

Light Catalytic Cracked Gas Oil
CAS No. 64741-59-9
(MRD-10-576)

PERFORMED FOR:

American Petroleum Institute
1220 L Street, NW
Washington, DC 20005-4070

PERFORMED AT:

ExxonMobil Biomedical Sciences, Inc.
1545 US Highway 22 East
Annandale, New Jersey 08801-3059

COMPLETION DATE: December 19, 2011

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APPROVAL SIGNATURES

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19 Dec 11
Date

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Environmental Toxicology & Fate
Laboratory Coordinator

19 Dec 11
Date

[redacted]
/Section Head, Environmental Sciences

19 Dec 2011
Date

The final report was accepted by the Sponsor

[redacted]
Sponsor Representative
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005-4070

15 Dec. 2011
Date

GLP COMPLIANCE STATEMENT

I hereby accept responsibility for the validity of these data and declare that to the best of my knowledge the study contained herein was performed under my supervision in compliance with the OECD Principles of Good Laboratory Practice, C(97) 186/Final, 1997 and the United States Environmental Protection Agency (USEPA) Toxic Substances Control Act, Good Laboratory Practice Standards, 40 CFR Part 792, 1989 with the exceptions listed below.

The elemental analysis of the test substance may not have been performed in a GLP compliant manner, since Quantitative Technologies Inc. (QTI) is not a GLP compliant facility. However, a standard, (Acetanilide), was employed to monitor the quality of the data. The values were within the standard limits.

Contaminant analysis of the water was not performed in a GLP compliant manner. Accutest® laboratory is accredited by the National Environmental Laboratory Accreditation Conference (NELAC). The analyses are performed using standard US EPA methods.

Both QTI and Accutest® have been audited by ExxonMobil Biomedical Sciences, Inc. using the ExxonMobil Quality Practices and Guidelines (QP & G v. 5.3).

The sponsor-supplied test substance analyses conducted by Intertek were not performed in a GLP compliant manner. These analyses were not conducted as part of the testing facility's protocol for this study.

It is unknown if the positive control substance (sodium benzoate) characterization and stability analyses were performed in a GLP compliant manner. The substance is supplied by Sigma Aldrich, an internationally recognized chemical supply company, and a certificate of analysis is included in the raw data and Appendix D.

This is not believed to have an adverse effect on the study results.

[redacted]

19 Dec 11
Date

Study Director
ExxonMobil Biomedical Sciences, Inc.
1545 US Highway 22 East
Annandale, NJ 08801-3059

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15 Dec. 2011
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1220 L Street, NW
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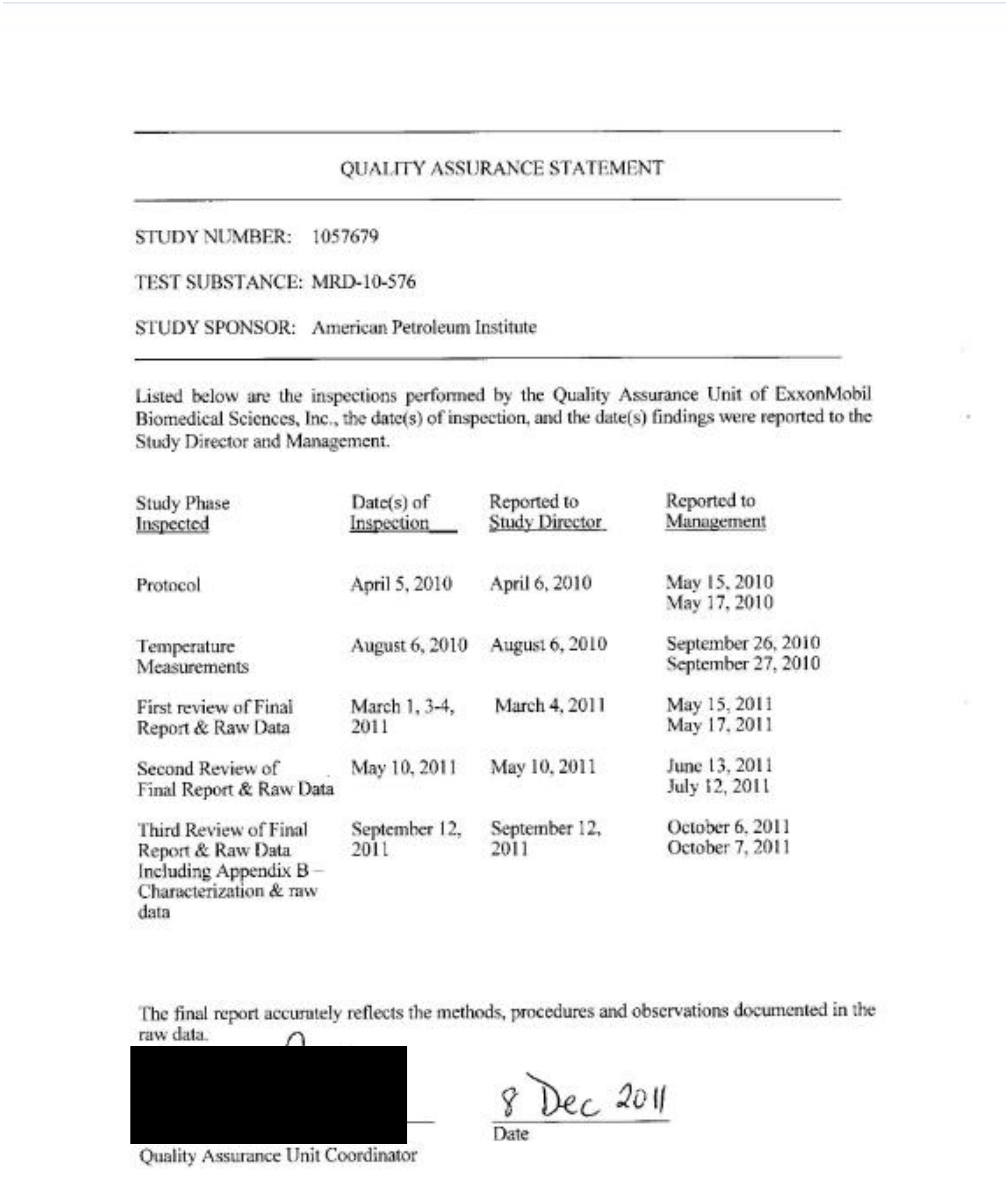


QUALITY ASSURANCE STATEMENT

STUDY NUMBER: 1057679

TEST SUBSTANCE: MRD-10-576

STUDY SPONSOR: American Petroleum Institute

Listed below are the inspections performed by the Quality Assurance Unit of ExxonMobil Biomedical Sciences, Inc., the date(s) of inspection, and the date(s) findings were reported to the Study Director and Management.

Study Phase Inspected	Date(s) of Inspection	Reported to Study Director	Reported to Management
Protocol	April 5, 2010	April 6, 2010	May 15, 2010 May 17, 2010
Temperature Measurements	August 6, 2010	August 6, 2010	September 26, 2010 September 27, 2010
First review of Final Report & Raw Data	March 1, 3-4, 2011	March 4, 2011	May 15, 2011 May 17, 2011
Second Review of Final Report & Raw Data	May 10, 2011	May 10, 2011	June 13, 2011 July 12, 2011
Third Review of Final Report & Raw Data Including Appendix B – Characterization & raw data	September 12, 2011	September 12, 2011	October 6, 2011 October 7, 2011

The final report accurately reflects the methods, procedures and observations documented in the raw data.

[redacted]

Quality Assurance Unit Coordinator

8 Dec 2011

Date

PERSONNEL

Study Director:

Sponsor Representative:

Section Head, Environmental Sciences:
(until July 1, 2011)

Section Head, Environmental Sciences:
(effective July 1, 2011)

Environmental Toxicology & Fate
Laboratory Coordinator:
(until to January 1, 2011)

Environmental Toxicology & Fate
Laboratory Coordinator:
(effective January 1, 2011)

Principal Investigator for Characterization:

Quality Assurance Unit Coordinator:

[redacted]

All personnel involved in the conduct of this study, except the sponsor, are/were located at the testing facility's address. The Sponsor Representative is located at the previously cited address.

SUMMARY

The aerobic biodegradation of a light catalytic cracked gas oil (CAS No. 64741-59-9) was measured in an OECD 301F test (manometric respirometry, OECD, 1992). A positive control treatment (sodium benzoate), a toxicity control treatment (light catalytic cracked gas oil in combination with the positive control substance), and an abiotic control treatment (light catalytic cracked oil with mercuric chloride) were included in the study design. The respirometer was operated within a temperature range of 21.1 – 22.9°C. Biodegradability was determined by measuring oxygen consumption in a test medium containing trace nutrients and inoculated with activated sludge supernatant. Light catalytic cracked gas oil, the positive control substance, the toxicity control and the abiotic control were evaluated at mean concentrations of 49.7, 48.7, 99.1, and 50.3 mg/l, respectively.

The average percent biodegradation on Day 28 of triplicate test systems containing light catalytic cracked gas oil was 56.32%. The study was extended and the test substance biodegraded to 61.23% on Day 47. A test substance shall be classified as readily biodegradable if the percent biodegradation reaches 60% within 28 days. According to Section 3 of Part 1: OECD Guidelines for Testing of Chemicals (OECD, 2006), the 10-day window criterion normally applied to pure substances should not be applied in interpreting results of testing mixtures for which a sequential biodegradation of structures is anticipated. Based on the guideline (OECD, 1992), the light catalytic cracked gas oil is inherently biodegradable.

The OECD guideline (OECD, 1992) validity requirement states that the difference between extremes of replicate biodegradation values should be less than 20% at the end of test and the positive control should achieve >60% biodegradation by Day 14. Further, the oxygen consumption of the inoculum blank should not exceed 60 mg/L in 28 days. This test passed all criteria set forth in the OECD guideline. Sodium benzoate biodegraded to >60% by Day 2 and the average of the cumulative oxygen consumed in the blank systems was 17.30 mg/L after 28 days. The difference between extremes for the light catalytic cracked gas oil after 28 days was 14.6% and 19.4% after 47 days. The difference between extremes for the toxicity control after 28 days was 7.1% and 7.8% after 47 days.

The guideline (OECD, 1992) states that a test substance will be considered inhibitory if the toxicity test systems, containing both the test and positive control substance, reach less than 25% biodegradation by Day 14. The toxicity control systems exceeded 25% on Day 3, which suggests that the test substance was not inhibitory at the tested concentration. The abiotic control replicates maintained 0% biodegradation for the entire test.

In conclusion, this light catalytic cracked gas oil did not achieve the 60% level of biodegradation within 28 days for classification as readily biodegradable. However, the test substance demonstrated a high capacity to be consumed by the microbial inoculum and is inherently biodegradable by guideline definition.

INTRODUCTION

Objective

This study was conducted for the sponsor in order to evaluate the potential of the test substance to biodegrade in an aerobic, aqueous environment for use in environmental hazard assessment.

Sponsor

American Petroleum Institute
1220 L Street, NW
Washington, DC 20005-4070

Testing Facilities

ExxonMobil Biomedical Sciences, Inc. (EMBSI)
Laboratory Operations
1545 US Highway 22 East
Annandale, New Jersey 08801-3059

Quantitative Technologies Inc. (QTI / Intertek) (Elemental Analysis Only)
P.O. Box 470
Salem Industrial Park, Bldg 5
Whitehouse, New Jersey 08888

Initial Characterization

12 July 2010

Study Initiation Date

28 June 2010

Experimental Start

14 July 2010

Experimental Termination

30 August 2010

Final Characterization

26 July 2011

INTRODUCTION (CONT'D)

Justification for Selection of Test System

Selection of the aerobic aquatic biodegradation test is based upon the OECD 301F Guideline for the Testing of Chemicals, (OECD, 1992). This test method is used to determine ready biodegradability by measuring oxygen consumption in a test system consisting of an activated sludge supernatant, test or control substance and a nutrient source. Further, activated sludge has historically been used to evaluate the persistence of chemicals in the environment.

Justification of Dosing Route

The test substance could possibly be found in aqueous solution in a wastewater treatment facility.

Compliance

The study was conducted in compliance with OECD (OECD, 1997) and USEPA Good Laboratory Practice (GLP) standards (USEPA, 1989) with the exceptions outlined on page 5 and was performed in agreement with the OECD 301F guideline (OECD, 1992) with the exceptions listed on page 18.

MATERIALS AND METHODS

Test Substance Identification

EMBSI Identification:	MRD-10-576
Sponsor Identification:	Light catalytic cracked gas oil Distillates (Petroleum)
CAS Number	64741-59-9
Supplier:	EPL Archives, Sterling, VA
Date Received:	24 June 2010
Expiration Date:	June 2015

<u>CAS Definition</u>: Distillates (petroleum) light catalytic cracked. A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C25 and boiling in the range of approximately 150 degrees C to 400 degrees C (302 degrees F to 752 degrees F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons (API, 1985).

Additional test substance information supplied by the Sponsor is attached in Appendix C.

Storage Conditions: The neat substance was stored at room temperature.

Characterization of Test Substance

The neat test substance was characterized and the stability determined by the testing facility both prior to and after completion of the study using the following analyses: Ultraviolet/Visible and Infrared Spectrophotometry, density, physical–state, miscibility in water, methanol and /or hexane and a GC-MS Total Ion chromatogram ("fingerprint") of the neat test substance. The GC-MS fingerprint is run against an ASTM hydrocarbon standard mixture. The ASTM D2887 standard is applied for higher boiling mixtures with compounds eluting between approximately n-octane (n-C8) and n-triacontane (n-C30). Due to the complex nature of the test substance, no reporting of specific hydrocarbon components was made. Instead, an area percent report was generated for both the pre- and post-test analysis to demonstrate stability of the test substance over the testing period. Documentation of characterization and stability assessment is maintained at the testing facility and reported in Appendix B.

The methods of synthesis, fabrication, and/or derivation of the test substance are maintained by the sponsor. The test substance, as received, was considered the “pure” substance.

MATERIALS AND METHODS (CONT'D)

Elemental Analysis (subcontracted)

An aliquot of the test substance was sent to Quantitative Technologies Inc. (QTI) for elemental analysis, specifically carbon, hydrogen, nitrogen and oxygen, see Appendix E. The Carbon, Hydrogen, Nitrogen, and Oxygen were determined using a Perkin-Elmer 2400 CHN Elemental Analyzer equipped with an oxygen accessory kit. For CHN, the analyzer used combustion to convert the sample elements to simple gases. Upon entering the analyzer, the sample was combusted in a pure oxygen environment. The product gases were separated under steady state conditions, and measured as a function of thermal conductivity. For oxygen, pyrolysis was used to convert the oxygen to carbon monoxide which was separated from the other pyrolozates under steady state conditions and measured as a function of thermal conductivity. This laboratory is not a GLP compliant facility and therefore may not have performed the analysis in a GLP compliant manner. However, a standard (Acetanilide) was employed to monitor the quality of the data. The standard data met acceptability criteria. QTI has been audited by ExxonMobil Biomedical Sciences, Inc. using the ExxonMobil Quality Practices and Guidelines (QP & G v. 5.3).

Positive Control Substance

Substance Identification: Sodium benzoate, 99%
Manufacturer: Sigma-Aldrich Company
Lot No. 11007MB
Expiration date: 26 June 2011
Storage Condition: The neat substance was stored at room temperature.

Characterization: The documentation of the stability, identity, solubility, purity, strength and composition or other characteristics which appropriately identify the positive control substance was provided by the manufacturer. It is unknown if the characterization and stability analysis was performed in a GLP compliant manner. The Certificate of Analysis sheet can be found in the raw data and Appendix D.

Dilution Water

Glass distilled water (gdH_2O) was used as the dilution water. The glass distilled water was prepared from UV sterilized deionized well water that was treated and distributed throughout the testing facility via PVC and stainless steel pipes. The feed water for the deionized water system was analyzed by Accutest®, 2235 Route 130, Dayton, New Jersey 08810. Results of the water analyses are maintained at the testing facility. There were no known contaminants in the water believed to be present at levels that may have interfered with this study. Contaminant analysis of the water was not performed in a GLP compliant manner. It is believed that this did not have an adverse effect on the study results. The laboratory is accredited by the National Environmental Laboratory Accreditation Conference (NELAC) and has been audited by ExxonMobil Biomedical Sciences, Inc. using the Quality Practices and Guidelines (QP & G v. 5.3). The analyses were performed using standard US EPA methods.

MATERIALS AND METHODS (CONT'D)

Inoculum Preparation

Fresh activated sludge was obtained from the Somerset-Raritan Valley Sewage Authority, Bridgewater, New Jersey on July 13, 2010 on Day -1 of the test. This treatment facility was selected because it deals predominantly with domestic sewage as specified in the guideline. There were no known contaminants in the fresh activated sludge believed to be present at levels high enough to have interfered with this study.

One half gallon of sludge was allowed to settle and approximately two hundred milliliters of supernatant was removed and discarded, in order to achieve the required 3-5 g/L total suspended solids (TSS) in the sludge. Duplicate 10 ml aliquots of the activated sludge were filtered through pre-weighed Whatman 934-AH filters in a Buchner funnel and vacuum flask set up. The filters were placed in an aluminum pan and dried in an oven for one hour at 103°C. After cooling the filters in a dessicator, the filters were re-weighed and the mean total suspended solids concentration was determined to be 3.74 g/L.

Approximately one liter of the sludge was homogenized in a blender for two minutes at low speed. The homogenated sample was allowed to settle in a one liter graduated cylinder for sixty-five minutes, after which the supernatant was decanted (avoiding carry-over of sludge solids). An aliquot of the supernatant was used to determine microbial activity using the Easicult®-TTC dip slides Lot No. 133817. This was accomplished by removing the agar stick from the culturing tube, and dipping the agar into the supernatant aliquot. Excess supernatant was blotted off with a clean paper towel, and the agar stick was then placed back into the culture tube. The whole unit was placed into a dark incubator for 48 hours at 20 ± 1°C. Initial and final temperatures were recorded with a calibrated mercury thermometer. Based on comparison of the density of colonies growing on the agar with the model density chart provided by the supplier, the microbial activity was determined to be 10^5 CFU/mL. The remaining decanted sludge supernatant was used for final preparation of the test medium on Day -1.

MATERIALS AND METHODS (CONT'D)

Test Medium Preparation

The test medium was prepared one day before the test began. Twenty-four liters of glass distilled water was collected in a carboy, and 552 mL of glass distilled water was removed to obtain a final volume of twenty-four liters after addition of the mineral solutions and inoculum. The following mineral salts and inoculum totaling 552 mL were added to the carboy:

mL per liter of glass distilled water	Solution
1.0	Magnesium sulfate, 2.25% (BDH, Lot# 8302) Exp: 31 October 2010
1.0	Calcium chloride, 2.75% (BDH, Lot# 0021105) Exp: 28 Feb 2011
10.0	Phosphate buffer, pH 7.2 (BDH, Lot# 0030918) Exp: 30 Mar 2011
1.0	Ferric chloride, 0.025% (BDH, Lot# 8338) Exp 30 Nov 2010
10.0	Activated sludge supernatant

The solution in the carboy was aerated with CO_2-free air (air passed through an Ascarite II column to remove CO_2), for approximately 25.5 hours.

There were no known contaminants in the solutions believed to be present at levels that may have interfered with the study. All solutions were refrigerated when not in use.

Positive Control Solution

Positive control substance stock solution: A stock solution of sodium benzoate at a concentration of approximately 10,000 mg/L was prepared in glass distilled water. A Shimadzu Total Organic Carbon (TOC) analyzer determined the actual carbon content of the sodium benzoate stock solution. The instrument employs combustion technique with a nondispersive infrared (NDIR) detector. TOC results are contained in Appendix A. The stock solution was refrigerated when not in use.

MATERIALS AND METHODS (CONT'D)

Preparation of the Test Systems

The test system was considered as one or any combination of the following in a flask:

- Test Substance, Positive Control Substance, Abiotic Control Substance, or a Combination of Test and Control Substances with test medium
- Test Medium (containing the following; Mineral Salt Solutions, Activated Sludge Supernatant, and Glass Distilled Water)

Test systems were prepared as follows:

Test System 1L Respirometer Flask		Amount of Neat Test Substance Added (mg)	Amount of 9734.48 mg/L[1] Sodium Benzoate Stock Solution Added (mL)	Test Medium (Liter)
Blank	Rep 1	---	---	1.0
	Rep 2	---	---	1.0
	Rep 3	---	---	1.0
Sodium Benzoate	Rep 1	---	5.0	1.0
	Rep 2	---	5.0	1.0
	Rep 3	---	5.0	1.0
MRD-10-576 (Light catalytic cracked gas oil)	Rep 1	50.4	---	1.0
	Rep 2	49.6	---	1.0
	Rep 3	49.2	---	1.0
MRD-10-576 w/ Sodium benzoate (Toxicity control)	Rep 1	51.8 (total 100.5)	5.0	1.0
	Rep 2	49.0 (total 97.7)	5.0	1.0
MRD-10-576 w/ Mercuric chloride (Abiotic control)	Rep 1	52.1	---	1.0
	Rep 2	48.5	---	1.0

[1] Reference concentration was calculated from the TOC value.

All test containers used in this study were uniquely identified as to appropriate composition, i.e., Study #, Blank -1, 2, 3; MRD-10-576 - 1, 2, 3, etc. All glassware was washed with ChemSolv® and then rinsed with glass distilled water to remove any residual organic carbon. All glassware was inspected to ensure cleanliness. The manometric cells were rinsed with two portions of acetone, filled with soapy water and allowed to stand for a few hours. The cells were then rinsed with glass distilled water followed by acetone then finally air dried. A set of blank and positive control test systems were shared for this study and Study # 1057779. Both studies ran concurrently and in an identical manner.

MATERIALS AND METHODS (CONT'D)

Preparation of the Test Systems (Cont'd)

Sodium benzoate was administered to the respective test systems by adding 5.0 mL aliquot of 9734.48mg/L aqueous stock solution. Concentration of the sodium benzoate was calculated from the TOC value. Mercuric chloride was administered to the abiotic control by adding 5.0 ml aliquot of a 10,000mg/l aqueous solution. The test substance was weighed onto glass fiber filters and added into the test flasks, followed by the addition of one liter of test medium. Each flask was sealed immediately to avoid loss due to volatilization. The stirrers were activated, the equipment was checked to ensure no leaks were present, and the oxygen uptake measurements were initiated. No further attention was required other than taking the necessary readings and making daily checks during normal working hours to see that the correct temperature (22 ± 1°C) and adequate stirring were maintained. After 47 days, data collection was terminated, and the pH in each of these flasks was measured, using a Symphony meter.

EXPERIMENTAL PROCEDURE

Procedure Summary

The test procedure evaluated the ready biodegradability of the test and positive control substances by microorganisms in water. The consumption of oxygen was determined by measuring the quantity of oxygen (produced electrolytically) required to maintain constant gas volume in the respirometer flask, or from the change in volume or pressure (or a combination of the two) in the apparatus. Evolved carbon dioxide was absorbed in a solution of 10N sodium hydroxide (NaOH). The amount of oxygen taken up by the microbial population during biodegradation of the test or positive control substance (corrected for uptake by blank inoculum, run in parallel) is expressed as a percentage of theoretical oxygen demand (ThOD). The ThOD calculation is found in Appendix A. The test was performed in general agreement with the OECD[4] guideline with the following clarifications/exceptions:

Clarifications

1. The apparatus is an electrolytic respirometer, manufactured by Co-ordinated Environmental Service (Kent, England). The system is based on a proven oxygen generating process coupled to a sensitive manometric cell. The sample was placed in a sample flask, which was then sealed by a manometric cell/CO_2 trap and immersed in a temperature stabilized water bath. For the duration of the experiment, the sample was stirred by a magnetically coupled stirrer. As the biodegradation process progressed, the microorganisms consumed O_2 converting it to CO_2 during aerobic respiration. The CO_2 produced was absorbed by a solution of 10N NaOH in the CO_2 trap, which caused a net reduction in gas pressure within the sample flask. This pressure reduction was detected by the manometric cell and triggered the electrolytic process. The electrolytic process generated oxygen, which restored the pressure in the sample flask. The magnitude and duration of the electrolyzing current is proportional to the amount of oxygen supplied to the microorganisms.

2. Light catalytic cracked gas oil, the positive control substance, the toxicity control and the abiotic control were evaluated at a mean concentration of 49.7 (test substance), 48.7 (positive control), 99.1 (combined test substance and positive control) and 50.3 mg/L (test substance with mercuric chloride), respectively.

3. The blank, positive control and test substance were tested in triplicate. The toxicity control and the abiotic control were tested in duplicate.

4. Bias was minimized by preparing the test medium on a large volume basis. In addition, the test medium was aerated for approximately 24 hours to improve homogeneity and ensure random distribution of test organisms to all test systems. The initial pH of individual systems was not determined due to the poor solubility of the test substance in water; the pH of the test medium was 7.37.

EXPERIMENTAL PROCEDURE (CONT'D)

Clarifications (Cont'd)

5. An aqueous stock solution was not prepared for the test substance because of the low water solubility. No concentration verification was performed since the test substance is poorly soluble in water.

6. Dissolved organic carbon (DOC) analysis was not performed due to the poor solubility of the test substance.

Exceptions

1. Test medium was prepared on a large volume basis, aerated and aliquoted into each test container, instead of preparing individual test systems.

2. The commercial phosphate buffer, used to prepare the test medium, had a pH value of 7.2 rather than 7.4. The phosphate buffer, purchased from BDH, has been approved by the American Public Health Association (APHA) for use in the Biological Oxygen Demand (BOD) analysis. The BOD buffer has the same composition as the buffer used in the OECD guideline (OECD, 1992).

3. The inoculum was mixed for 2 minutes in a blender at low speed, instead of medium speed.

RESULTS AND DISCUSSION

This study was conducted in agreement with the OECD 301F guideline (OECD, 1992). The average percent biodegradation of triplicate test systems of the light catalytic cracked gas oil was determined to be 56.32% over a 28 day testing period. The testing period was extended to see if the test substance had the ability to further degrade. The test substance biodegraded to 61.23% on Day 47. Based on the OECD guideline (OECD, 1992), this sample of light catalytic cracked gas oil can be considered inherently biodegradable.

A toxicity control (test substance in combination with the positive control substance) was also studied at a mean combined concentration of 99.1 mg/L. The guideline (OECD, 1992) states that a test substance will be considered inhibitory if the toxicity test systems, containing both the test and positive control substance, reach less than 25% biodegradation by Day 14. The toxicity control systems exceeded 25% on Day 3, which suggests that the test substance was not inhibitory at the tested concentration of approximately 50 mg/L. The abiotic control systems maintained 0% biodegradation throughout the study.

The OECD guideline (OECD, 1992) validity requirement states that the difference between extremes of replicate biodegradation values should be less than 20% at the end of test and the positive control should achieve >60% biodegradation by Day 14. Further, the oxygen consumption of the inoculum blank should not exceed 60 mg/L in 28 days. This test passed all validity criteria set forth in the OECD guideline. Sodium benzoate biodegraded to >60% by Day 2 and the average of the cumulative oxygen consumed in the blank systems was 17.30 mg/L after 28 days. The difference between extremes for the light catalytic cracked gas oil after 28 days was 14.6% and 19.4% after 47 days. The difference between extremes for the toxicity control after 28 days was 7.1% and 7.8% after 47 days.

The percent biodegradation results and mg oxygen consumed for each test system are reported in Tables 1 and 2, respectively. A graphical illustration representing the mean biodegradation and the mg oxygen consumed by the test systems is reported in Figures 1 and 2, respectively.

RESULT AND DISCUSSION (CONT'D)

The table below summarizes the biodegradation data.

Test System	Rep #	Day 28 data		Day 47 data		Final pH On Day 47
		% Biodegradation	Mean (SD)	% Biodegradation	Mean (SD)	
Sodium Benzoate	1 2 3	85.79 94.03 91.55	90.46 (4.23)	85.79 94.03 91.55	90.46 (4.23)	7.26 7.27 7.25
MRD-10-576 (Light catalytic cracked gas oil)	1 2 3	60.55 56.11 52.31	56.32 (4.12)	68.82 58.24 56.64	61.23 (6.62)	7.12 7.14 7.10
MRD-10-576 w/Sodium Benzoate (Toxicity control)	1 2	64.43 69.17	66.80 (3.35)	68.34 73.91	71.13 (3.94)	7.26 7.31
MRD-10-576 w/Mercuric chloride (Abiotic control)	1 2	0 0	0	0 0	0	7.26 7.25

The mean percent biodegradation, percent biodegradation for systems with sodium benzoate and ThOD calculations were written in Microsoft Excel®; therefore, some rounding differences may be noted. Although the Excel® program reports rounded values, internal rounding was not performed to calculate the final results. The percent biodegradation for systems without sodium benzoate were calculated using the respirometer software.

CONCLUSION

Light catalytic cracked gas oil (CAS No. 64741-59-9) achieved a 28-day biodegradation of 56.32% in an OECD 301F test (manometric respirometry, OECD, 1992). The test was extended to 47 days with biodegradation attaining a level of 61.23%. While this test substance did not pass the criteria for ready biodegradation classification, the test substance showed a high capacity for biodegradability in this study. This light catalytic cracked gas oil can be considered inherently biodegradable by the OECD guideline (OECD, 1992) definition.

RECORDS

All appropriate materials, methods and experimental measurements required by the protocol have been recorded and documented in the raw data.

The protocol, final report, raw data or computer-generated listings of raw data, supporting study documentation and a non-study specific neat test substance sample are maintained in the archives of the testing facility.

REFERENCES

Organization for Economic Cooperation and Development, Principles of Good Laboratory Practice, C(97) 186/Final, 1997.

United States Environmental Protection Agency (USEPA) Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards, 40 CFR Part 792, 1989.

Organization for Economic Cooperation and Development (OECD), Guidelines for the Testing of Chemicals, Section 3, Introduction. (Adopted 23 March 2006).

Organization for Economic Cooperation and Development (OECD), Guideline for Testing of Chemicals, Ready Biodegradability, 301F Manometric Respirometry Test (1992).

API. Petroleum process stream terms included in the chemical substances inventory under the Toxic Substances Control Act (TSCA). American Petroleum Institute, Washington, DC. February, 1985. 40 pp.

TABLE 1-1

PERCENT BIODEGRADATION RESULTS

DAY OF TEST	Sodium Benzoate					MRD-10-576 w/ Sodium Benzoate			
	Rep 1	Rep 2	Rep 3	Mean	SD	Rep 1	Rep 2	Mean	SD
1	29.05	29.36	28.46	28.96	0.46	12.73	13.14	12.94	0.29
2	60.22	61.52	61.60	61.11	0.77	23.21	24.94	24.08	1.23
3	73.46	75.59	75.59	74.88	1.23	28.68	30.45	29.57	1.26
4	79.11	81.59	81.31	80.67	1.36	31.55	35.43	33.49	2.74
5	82.96	86.46	86.37	85.26	2.00	45.42	47.87	46.64	1.73
6	84.45	88.91	88.54	87.30	2.48	51.29	52.82	52.05	1.08
7	86.13	91.95	91.48	89.86	3.23	52.64	54.16	53.40	1.08
8	85.15	90.61	90.04	88.60	3.00	52.20	53.97	53.09	1.25
9	84.59	90.69	89.96	88.41	3.33	52.57	54.82	53.70	1.59
10	86.14	92.70	91.77	90.20	3.55	53.49	55.94	54.71	1.73
11	87.40	94.03	92.49	91.31	3.47	54.25	56.92	55.59	1.89
12	87.44	94.28	92.65	91.46	3.57	55.03	58.25	56.64	2.28
13	87.21	94.45	92.46	91.37	3.74	55.80	59.29	57.54	2.47
14	86.68	94.35	92.19	91.07	3.95	56.25	60.45	58.35	2.97
15	86.00	93.86	91.51	90.45	4.03	56.56	61.17	58.87	3.26
16	85.79	94.03	91.55	90.46	4.23	57.03	61.66	59.34	3.28
17	85.79	94.03	91.55	90.46	4.23	57.75	62.34	60.04	3.24
18	85.79	94.03	91.55	90.46	4.23	58.38	63.09	60.74	3.33
19	85.79	94.03	91.55	90.46	4.23	59.17	63.99	61.58	3.41
20	85.79	94.03	91.55	90.46	4.23	59.90	64.82	62.36	3.48
21	85.79	94.03	91.55	90.46	4.23	60.51	65.49	63.00	3.53
22	85.79	94.03	91.55	90.46	4.23	61.09	66.09	63.59	3.54
23	85.79	94.03	91.55	90.46	4.23	61.68	66.69	64.19	3.55
24	85.79	94.03	91.55	90.46	4.23	62.27	67.30	64.78	3.56
25	85.79	94.03	91.55	90.46	4.23	62.84	67.82	65.33	3.52
26	85.79	94.03	91.55	90.46	4.23	63.41	68.43	65.92	3.54
27	85.79	94.03	91.55	90.46	4.23	63.93	68.80	66.36	3.45
28	85.79	94.03	91.55	90.46	4.23	64.43	69.17	66.80	3.35
29	85.79	94.03	91.55	90.46	4.23	65.02	69.48	67.25	3.15
30	85.79	94.03	91.55	90.46	4.23	65.47	69.77	67.62	3.05
31	85.79	94.03	91.55	90.46	4.23	65.79	69.93	67.86	2.93
32	85.79	94.03	91.55	90.46	4.23	66.17	70.23	68.20	2.87
33	85.79	94.03	91.55	90.46	4.23	66.55	70.53	68.54	2.81
34	85.79	94.03	91.55	90.46	4.23	66.81	70.75	68.78	2.79
35	85.79	94.03	91.55	90.46	4.23	67.13	71.06	69.09	2.78
36	85.79	94.03	91.55	90.46	4.23	67.32	71.35	69.33	2.85
37	85.79	94.03	91.55	90.46	4.23	67.57	71.80	69.69	2.99
38	85.79	94.03	91.55	90.46	4.23	67.76	72.11	69.93	3.07
39	85.79	94.03	91.55	90.46	4.23	67.96	72.48	70.22	3.20
40	85.79	94.03	91.55	90.46	4.23	68.08	72.78	70.43	3.32
41	85.79	94.03	91.55	90.46	4.23	68.21	73.01	70.61	3.39
42	85.79	94.03	91.55	90.46	4.23	68.28	73.23	70.75	3.51
43	85.79	94.03	91.55	90.46	4.23	68.34	73.46	70.90	3.62
44	85.79	94.03	91.55	90.46	4.23	68.34	73.61	70.97	3.72
45	85.79	94.03	91.55	90.46	4.23	68.34	73.68	71.01	3.78
46	85.79	94.03	91.55	90.46	4.23	68.34	73.83	71.09	3.88
47	85.79	94.03	91.55	90.46	4.23	68.34	73.91	71.13	3.94

TABLE 1-1 (CONT'D)

PERCENT BIODEGRADATION RESULTS (CONT'D)

DAY	MRD-10-576 w/ Mercuric Chloride				MRD-10-576				
OF TEST	Rep 1	Rep 2	Mean	SD	Rep 1	Rep 2	Rep 3	Mean	SD
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	3.33	4.98	5.95	4.75	1.32
4	0.00	0.00	0.00	0.00	18.82	14.74	13.96	15.84	2.61
5	0.00	0.00	0.00	0.00	34.42	34.35	32.08	33.62	1.33
6	0.00	0.00	0.00	0.00	38.46	37.32	35.63	37.14	1.42
7	0.00	0.00	0.00	0.00	39.14	37.41	36.25	37.60	1.45
8	0.00	0.00	0.00	0.00	38.80	36.40	35.77	36.99	1.60
9	0.00	0.00	0.00	0.00	40.51	37.71	36.77	38.33	1.95
10	0.00	0.00	0.00	0.00	43.59	40.56	39.22	41.12	2.24
11	0.00	0.00	0.00	0.00	44.99	41.47	42.38	42.95	1.83
12	0.00	0.00	0.00	0.00	46.09	42.13	43.48	43.90	2.01
13	0.00	0.00	0.00	0.00	46.96	42.75	44.21	44.64	2.14
14	0.00	0.00	0.00	0.00	47.79	43.43	44.79	45.34	2.23
15	0.00	0.00	0.00	0.00	48.54	43.93	45.40	45.96	2.35
16	0.00	0.00	0.00	0.00	49.47	44.62	45.99	46.69	2.50
17	0.00	0.00	0.00	0.00	50.57	45.68	46.85	47.70	2.55
18	0.00	0.00	0.00	0.00	51.48	46.96	47.70	48.71	2.42
19	0.00	0.00	0.00	0.00	52.58	48.55	48.45	49.86	2.36
20	0.00	0.00	0.00	0.00	53.68	50.25	49.11	51.01	2.38
21	0.00	0.00	0.00	0.00	54.96	51.85	49.75	52.19	2.62
22	0.00	0.00	0.00	0.00	56.15	52.91	50.21	53.09	2.97
23	0.00	0.00	0.00	0.00	57.25	53.66	50.68	53.86	3.29
24	0.00	0.00	0.00	0.00	58.07	54.19	51.12	54.46	3.48
25	0.00	0.00	0.00	0.00	58.71	54.72	51.44	54.96	3.64
26	0.00	0.00	0.00	0.00	59.45	55.25	51.77	55.49	3.85
27	0.00	0.00	0.00	0.00	60.00	55.68	51.99	55.89	4.01
28	0.00	0.00	0.00	0.00	60.55	56.11	52.31	56.32	4.12
29	0.00	0.00	0.00	0.00	61.00	56.54	52.53	56.69	4.24
30	0.00	0.00	0.00	0.00	61.46	56.96	52.74	57.05	4.36
31	0.00	0.00	0.00	0.00	61.97	57.18	52.85	57.33	4.56
32	0.00	0.00	0.00	0.00	62.52	57.39	53.07	57.66	4.73
33	0.00	0.00	0.00	0.00	63.09	57.71	53.88	58.23	4.63
34	0.00	0.00	0.00	0.00	63.42	57.92	54.09	58.48	4.69
35	0.00	0.00	0.00	0.00	63.70	58.13	54.31	58.71	4.72
36	0.00	0.00	0.00	0.00	63.97	58.13	54.92	59.01	4.59
37	0.00	0.00	0.00	0.00	64.15	58.24	55.24	59.21	4.53
38	0.00	0.00	0.00	0.00	64.34	58.24	55.57	59.38	4.50
39	0.00	0.00	0.00	0.00	64.57	58.24	55.78	59.53	4.53
40	0.00	0.00	0.00	0.00	64.79	58.24	56.18	59.74	4.50
41	0.00	0.00	0.00	0.00	64.96	58.24	56.29	59.83	4.55
42	0.00	0.00	0.00	0.00	65.12	58.24	56.40	59.92	4.60
43	0.00	0.00	0.00	0.00	65.29	58.24	56.40	59.98	4.69
44	0.00	0.00	0.00	0.00	66.82	58.24	56.64	60.57	5.47
45	0.00	0.00	0.00	0.00	67.00	58.24	56.64	60.63	5.58
46	0.00	0.00	0.00	0.00	67.10	58.24	56.64	60.66	5.63
47	0.00	0.00	0.00	0.00	68.82	58.24	56.64	61.23	6.62

TABLE 2-1

MG OXYGEN CONSUMED RESULTS

DAY	BLANK					Sodium Benzoate				
OF TEST	Rep 1	Rep 2	Rep 3	Mean	SD	Rep 1	Rep 2	Rep 3	Mean	SD
1	0.00	0.00	0.00	0.00	0.00	23.62	23.87	23.14	23.54	0.37
2	0.00	0.00	0.00	0.00	0.00	48.96	50.02	50.08	49.69	0.63
3	0.00	0.00	0.25	0.08	0.14	59.81	61.54	61.54	60.96	1.00
4	0.23	0.56	1.00	0.60	0.39	64.91	66.93	66.70	66.18	1.11
5	1.52	1.77	2.42	1.90	0.46	69.35	72.20	72.12	71.22	1.62
6	3.44	4.10	4.71	4.08	0.64	72.74	76.37	76.07	75.06	2.01
7	7.00	7.77	9.06	7.94	1.04	77.97	82.70	82.32	81.00	2.63
8	11.79	12.93	13.87	12.86	1.04	82.09	86.53	86.07	84.90	2.44
9	14.91	14.93	14.56	14.80	0.21	83.57	88.53	87.94	86.68	2.71
10	15.33	15.10	14.56	15.00	0.40	85.03	90.36	89.61	88.33	2.89
11	15.74	15.43	14.56	15.24	0.61	86.30	91.69	90.44	89.48	2.82
12	16.16	15.89	14.56	15.54	0.86	86.63	92.19	90.86	89.89	2.90
13	16.58	16.56	14.56	15.90	1.16	86.80	92.69	91.07	90.19	3.04
14	17.20	17.22	14.56	16.33	1.53	86.80	93.03	91.28	90.37	3.21
15	18.03	18.06	14.56	16.88	2.01	86.80	93.19	91.28	90.42	3.28
16	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
17	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
18	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
19	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
20	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
21	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
22	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
23	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
24	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
25	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
26	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
27	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
28	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
29	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
30	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
31	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
32	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
33	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
34	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
35	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
36	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
37	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
38	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
39	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
40	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
41	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
42	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
43	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
44	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
45	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
46	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44
47	18.45	18.89	14.56	17.30	2.38	87.05	93.75	91.73	90.84	3.44

TABLE 2-1 (CONT'D)

MG OXYGEN CONSUMED RESULTS (CONT'D)

DAY	MRD-10-576 w/ Sodium Benzoate				MRD-10-576 w/ Mercuric Chloride				MRD-10-576				
OF TEST	Rep 1	Rep 2	Mean	SD	Rep 1	Rep 2	Mean	SD	Rep 1	Rep 2	Rep 3	Mean	SD
1	29.07	29.13	29.10	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	52.98	55.31	54.15	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	65.56	67.61	66.59	1.45	0.00	0.00	0.00	0.00	5.39	7.89	9.33	7.54	1.99
4	72.64	79.16	75.90	4.61	0.00	0.00	0.00	0.00	30.57	23.70	22.30	25.52	4.43
5	105.60	108.04	106.82	1.73	0.00	0.00	0.00	0.00	56.73	55.75	51.77	54.75	2.63
6	121.18	121.20	121.19	0.01	1.23	0.00	0.62	0.87	65.33	62.58	59.48	62.46	2.93
7	128.12	128.03	128.08	0.06	1.23	0.00	0.62	0.87	70.28	66.58	64.31	67.06	3.01
8	132.05	132.53	132.29	0.34	1.23	0.00	0.62	0.87	74.66	69.91	68.47	71.01	3.24
9	134.82	136.36	135.59	1.09	1.48	0.00	0.74	1.05	79.32	73.91	71.97	75.07	1.00
10	137.11	139.03	138.07	1.36	1.48	0.00	0.74	1.05	84.42	78.57	75.97	79.65	4.33
11	139.11	141.46	140.29	1.66	1.48	0.00	0.74	1.05	86.90	80.24	81.13	82.76	3.62
12	141.17	144.69	142.93	2.49	1.92	0.00	0.96	1.36	88.94	81.57	83.13	84.55	3.88
13	143.30	147.36	145.33	2.87	2.50	0.00	1.25	1.77	90.69	82.90	84.63	86.07	4.09
14	144.75	150.36	147.56	3.97	2.50	0.00	1.25	1.77	92.44	84.40	85.97	87.60	4.26
15	146.02	152.52	149.27	4.60	2.50	0.00	1.25	1.77	94.19	85.74	87.47	89.13	4.46
16	147.50	154.02	150.76	4.61	2.50	0.00	1.25	1.77	96.09	87.24	88.80	90.71	4.72
17	149.15	155.52	152.34	4.50	2.50	0.00	1.25	1.77	97.84	88.90	90.13	92.29	4.85
18	150.59	157.19	153.89	4.67	2.92	0.00	1.46	2.06	99.29	90.90	91.46	93.88	4.69
19	152.40	159.19	155.80	4.80	3.98	0.00	1.99	2.81	101.04	93.40	92.63	95.69	4.65
20	154.06	161.02	157.54	4.92	3.98	0.00	1.99	2.81	102.79	96.07	93.65	97.50	4.74
21	155.44	162.52	158.98	5.01	3.98	0.00	1.99	2.81	104.83	98.56	94.65	99.35	5.14
22	156.77	163.85	160.31	5.01	3.98	0.00	1.99	2.81	106.73	100.23	95.36	100.77	5.70
23	158.12	165.18	161.65	4.99	3.98	0.00	1.99	2.81	108.48	101.40	96.09	101.99	6.22
24	159.46	166.52	162.99	4.99	4.10	0.00	2.05	2.90	109.79	102.23	96.77	102.93	6.54
25	160.77	167.68	164.23	4.89	4.71	0.00	2.36	3.33	110.81	103.06	97.27	103.71	6.79
26	162.08	169.02	165.55	4.91	4.83	0.00	2.42	3.42	111.98	103.90	97.79	104.56	7.12
27	163.25	169.85	166.55	4.67	4.83	0.00	2.42	3.42	112.85	104.56	98.13	105.18	7.38
28	164.41	170.68	167.55	4.43	4.83	0.00	2.42	3.42	113.73	105.25	98.63	105.87	7.57
29	165.75	171.35	168.55	3.96	4.83	0.00	2.42	3.42	114.45	105.92	98.96	106.44	7.76
30	166.77	172.01	169.39	3.71	5.73	0.00	2.87	4.05	115.18	106.58	99.29	107.02	7.95
31	167.50	172.35	169.93	3.43	6.21	0.00	3.11	4.39	116.00	106.92	99.46	107.46	8.28
32	168.37	173.01	170.69	3.28	6.21	0.00	3.11	4.39	116.87	107.25	99.81	107.98	8.55
33	169.24	173.68	171.46	3.14	6.21	0.00	3.11	4.39	117.79	107.75	101.06	108.87	8.42
34	169.83	174.18	172.01	3.08	6.21	0.00	3.11	4.39	118.31	108.08	101.40	109.26	8.52
35	170.56	174.85	172.71	3.03	6.21	0.00	3.11	4.39	118.74	108.42	101.73	109.63	8.57
36	170.99	175.51	173.25	3.20	6.21	0.00	3.11	4.39	119.18	108.42	102.69	110.10	8.37
37	171.58	176.51	174.05	3.49	6.21	0.00	3.11	4.39	119.47	108.58	103.19	110.41	8.29
38	172.01	177.18	174.60	3.66	6.21	0.00	3.11	4.39	119.76	108.58	103.69	110.68	8.24
39	172.45	178.01	175.23	3.93	6.21	0.00	3.11	4.39	120.14	108.58	104.02	110.91	8.31
40	172.74	178.68	175.71	4.20	6.21	0.00	3.11	4.39	120.49	108.58	104.65	111.24	8.25
41	173.03	179.18	176.11	4.35	6.21	0.00	3.11	4.39	120.76	108.58	104.81	111.38	8.34
42	173.18	179.68	176.43	4.60	6.21	0.00	3.11	4.39	121.01	108.58	104.98	111.52	8.41
43	173.33	180.18	176.76	4.84	6.27	0.00	3.14	4.43	121.28	108.58	104.98	111.61	8.56
44	173.33	180.51	176.92	5.08	7.81	0.00	3.91	5.52	123.72	108.58	105.35	112.55	9.81
45	173.33	180.68	177.01	5.20	8.43	0.00	4.22	5.96	124.01	108.58	105.35	112.65	9.97
46	173.33	181.01	177.17	5.43	8.50	0.00	4.25	6.01	124.16	108.58	105.35	112.70	10.06
47	173.33	181.18	177.26	5.55	8.93	0.00	4.47	6.31	126.91	108.58	105.35	113.61	11.63

FIGURE 1

PERCENT BIODEGRADATION
(Average of Replicates)

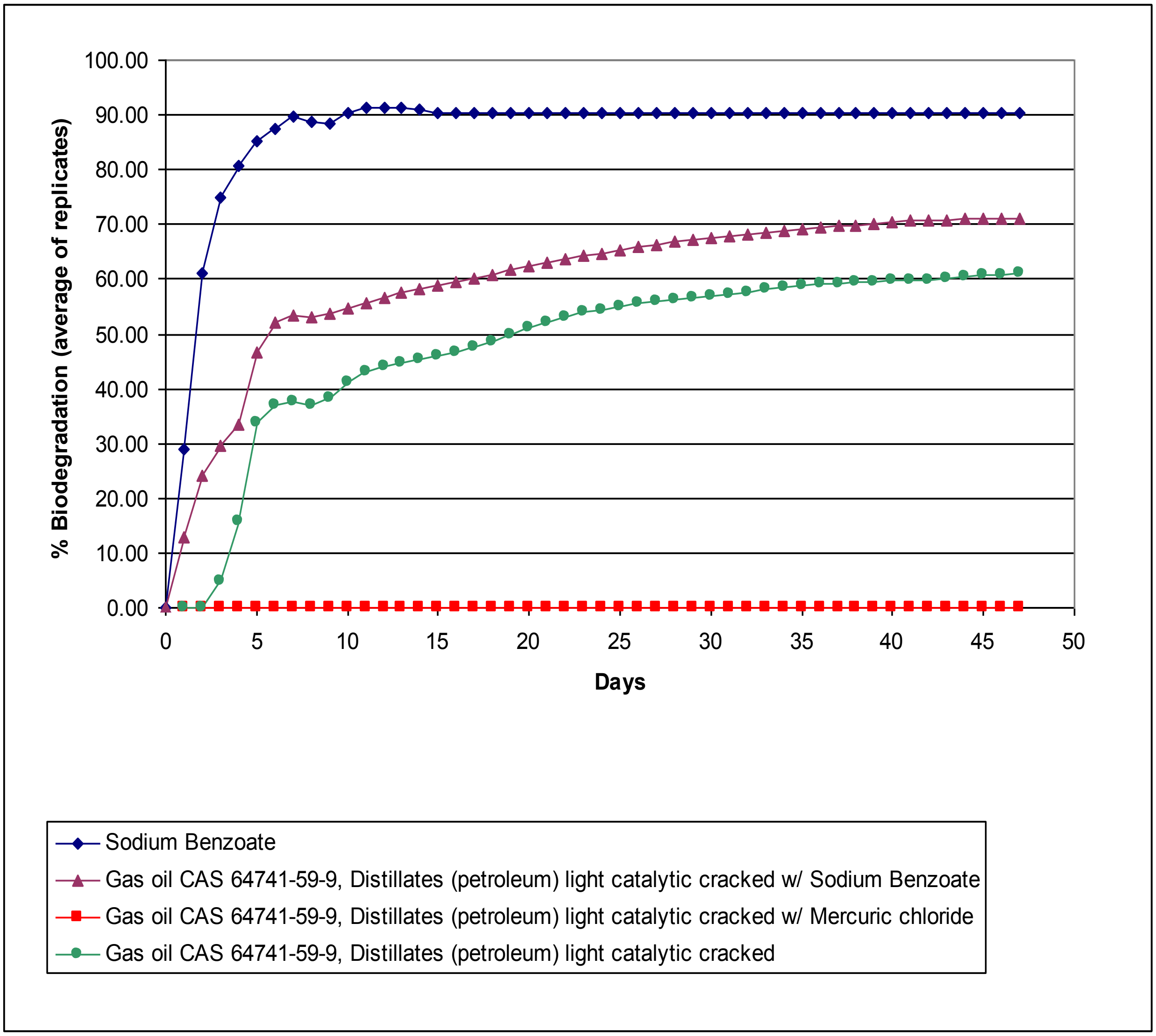
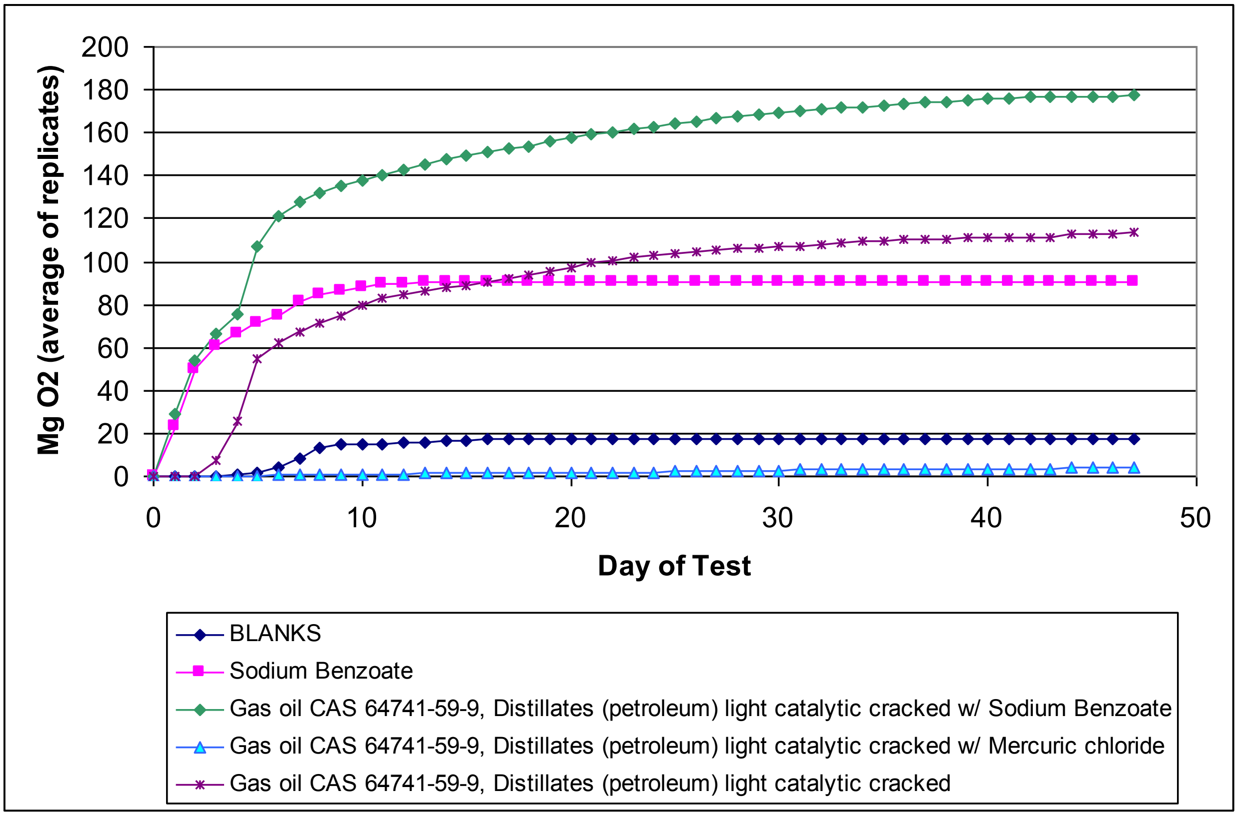


FIGURE 2

MG OXYGEN CONSUMED
(Average of Replicates)



APPENDIX A - CALCULATIONS

Theoretical Oxygen Demand (ThOD)

The empirical formula and the theoretical oxygen demand (ThOD) of the test substance was calculated from elemental analysis data (assuming 100 gram test substance). Sodium benzoate ThOD was calculated using the empirical formula and was determined to be 1.67 mg O_2/mg sodium benzoate. The ThOD calculations of the test and positive control substances were based on Annex IV of the OECD guideline (OECD, 1992).

TEST SUBSTANCE	% CARBON	% HYDROGEN	% OXYGEN	MOLE OF CARBON	MOLE OF HYDROGEN	MOLE OF OXYGEN	MOLE OF SODIUM	MOL. WT.	ThOD
Sodium Benzoate (SB)				7.00	5.00	2.00	1.00	144.11	1.67
MRD-10-576	89.02	9.26		7.41	9.19	0.00		98.28	3.16
MRD-10-576 W/ SB				14.41	14.19	2.00	1.00	242.39	2.27

%N of test substance will not be included in ThOD. Nitrogen value was less than 0.10% which will not affect the final ThOD value
% O of the test substance was <0.1%

MOLE = % ELEMENT (IN GRAMS)/ATOMIC WT OF ELEMENT)
AT. WT OF C = 12.011 G/MOLE
AT. WT OF H = 1.0079 G/MOLE
AT. WT OF O = 15.999 G/MOLE
AT. WT OF Na = 22.990 G/MOLE

MOL. WT. = (MOLE OF CARBON x AT. WT.)+(MOLE OF HYDROGEN x AT. WT.)+(MOLE OF OXYGEN x AT. WT.)+(MOLE OF SODIUM x AT. WT.)

ThOD (mg O2/mg Test Substance) = [16 x ((2 x NO. OF CARBON) + (0.5 x NO. OF HYDROGEN)+(0.5 x NO. OF SODIUM)) - (NO. OF OXYGEN))]/MOL. WT.

Due to the calculation process of EXCEL 2003 some rounding differences may be noted. This will not have an adverse affect on the integrity of the data.

Sodium Benzoate Concentration

The sodium benzoate stock solution concentration was determined to be 9734.48 mg sodium benzoate/L by dividing the solution total organic carbon (TOC) content (5646 mg carbon /L) by the percent carbon of sodium benzoate (58%). A 5 mL aliquot of the solution added to each test system contained 48.7 mg of sodium benzoate. The pH of the stock solution was 7.37.

Percent Biodegradation Values

Biodegradation results for the test and positive control substances were calculated as the net amount of oxygen (mg) consumed by the test system multiplied by a constant. The net amount of oxygen was calculated as the difference between the test system oxygen consumption (mg) and the average oxygen (mg) consumed by the blanks. The amount of oxygen consumed was recorded at each hourly interval for each test system using the CES aerobic respirometer. The constant is defined as the inverse of the product of the ThOD and the mg of substance added to that system multiplied by 100.

Percent biodegradation values were calculated using Microsoft Excel® and/or the respirometer software. The following parameters were used to calculate biodegradation for each system: the ThOD and the mass of the positive control or test substance added. The respirometer software generates the hourly logged oxygen uptake values. The following calculation was used to derive the percent biodegradation.

$$Constant = 100 \, \frac{1}{ThOD \; x \; mg \; test \; substance \; in \; vessel}$$

APPENDIX A – CALCULATIONS (CONT'D)

Percent Biodegradation Values (Cont'd)

$$\%\,\text{Percent Biodegradation} = (\text{mg } O_2 \text{ uptake by test substance} - \overline{X} \text{ mg } O_2 \text{ uptake by blank}) \times \text{constant}$$

The mg of oxygen consumed by the blank represents the average oxygen consumption for the triplicate test systems.

Difference of Extremes- % Biodegradation Replicates

Difference of Extremes were calculated using the highest replicate value subtracting the lowest replicate value, divided by the mean of the high and low replicate, multiplied by 100.

Difference of Extremes =((highest replicate value - lowest replicate value) / mean of high and low values) x 100

APPENDIX B – TEST SUBSTANCE CHARACTERIZATION

TEST SUBSTANCE CHARACTERIZATION

The light catalytic cracked gas oil (CAS No. 64741-59-9) was initially characterized on July 12, 2010. Analyses included Ultraviolet-Visible (UV-VIS) spectroscopy and Fourier Transform Infrared (FT-IR) spectroscopy, density and Gas chromatography–mass spectrometry (GC-MS) analysis. Stability of the neat test substance was confirmed by repeating these same analyses on July 26, 2011 after completion of this study.

UV-VIS spectra are presented in Figures UV-VIS-1 and UV-VIS-2 representing, the initial and final spectrum at concentrations of 17.8 ppm and 13.5 ppm, respectively. UV-VIS spectra were acquired on a Hewlett-Packard 8453 diode array UV-VIS spectrophotometer using a 1 cm quartz cell, a scan time of 0.5 seconds and resolution of 2 nm.

FT-IR spectra of the neat test substance are presented in Figures FTIR-1 and FTIR-2 representing the initial and final spectra. Initial and final FT-IR spectra were acquired on a Thermo Nicolet Avatar 360 FT-IR spectrometer with a KBr plate. The spectra were obtained with the following settings: resolution of 4 cm^{-1}, gain of 1 and scan number of 32.

The test substance was also characterized by GC-MS using a Hewlett-Packard HP5890 Series II gas chromatograph with 5972 mass selective detector. For comparison of relative retention times to a series of known hydrocarbons under the analytical conditions employed, MRD-10-576 was analyzed against an ASTM D2887 calibration mixture. Figures Total IonChromatogram-1 and Total Ion Chromatogram-2 represent the initial and final GC-MS total ion chromatograms, respectively. The test substance eluted as a complex mixture with numerous chromatographic components between retention times of approximately 17 and 27 minutes. This corresponds to bracketing by standard hydrocarbons n-dodecane (n-C12) and n-eicosane (n-C20) under the analytical conditions employed.

The test substance's initial and final density was measured at 20°C with an Anton Paar DMA 4500 Density/Specific gravity/Concentration meter. The initial density was measured as 0.9576 g/mL@20°C and final density was measured as 0.9578 g/mL@20°C. The test substance was observed to be a liquid under ambient laboratory conditions and immiscible in water and methanol but miscible in hexane.

Comparison of the initial and final analyses appeared to be substantially similar indicating the neat test substance was stable over the duration of the study period.

[redacted]

[redacted] Principal Investigator for Characterization (located at the testing facility)

9 Nov 2011
Date

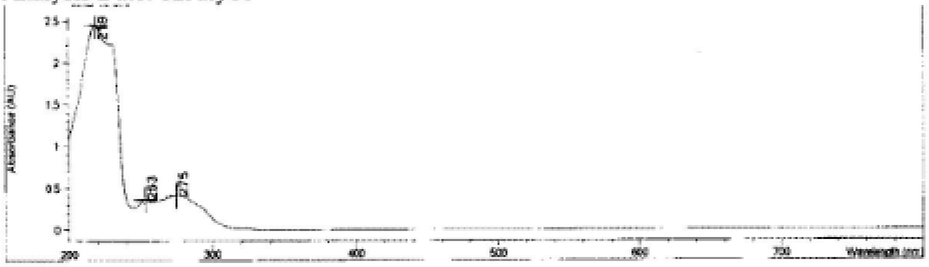
APPENDIX B – TEST SUBSTANCE CHARACTERIZATION (CONT'D)

TEST SUBSTANCE CHARACTERIZATION (CONT'D)

UV-VIS SPECTRA

Figure: UV-VIS-1
Initial

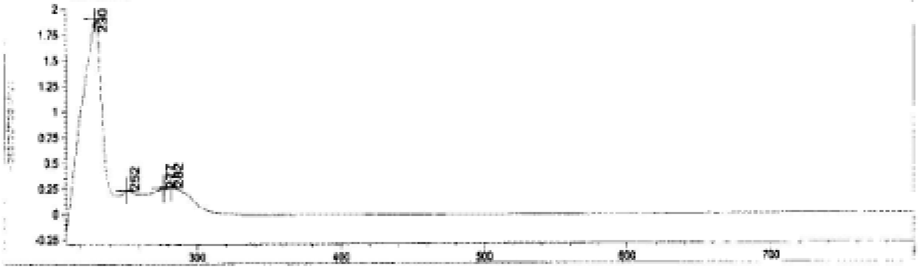
Initial Characterization MRD-10-576 17.8 ppm solution in hexane
Analysis Date: 12July10



Peak 219nm Absorbance = 2.4373
Peak 253nm Absorbance = 0.3603
Peak 275nm Absorbance = 0.408

Figure: UV-VIS-2
Final

Final Characterization MRD-10-576 13.5 ppm solution in hexane



Analysis Date: 26Jul11

Peak 230nm Absorbance = 1.90510
Peak 277nm Absorbance = 0.24967
Peak 252nm Absorbance = 0.22738
Peak 282nm Absorbance = 0.26198

APPENDIX B – TEST SUBSTANCE CHARACTERIZATION (CONT'D)

TEST SUBSTANCE CHARACTERIZATION (CONT'D)

FT-IR SPECTRA

Figure: FTIR-1
Initial

Initial Characterization MRD-10-576 Analysis Date: 12Jul10

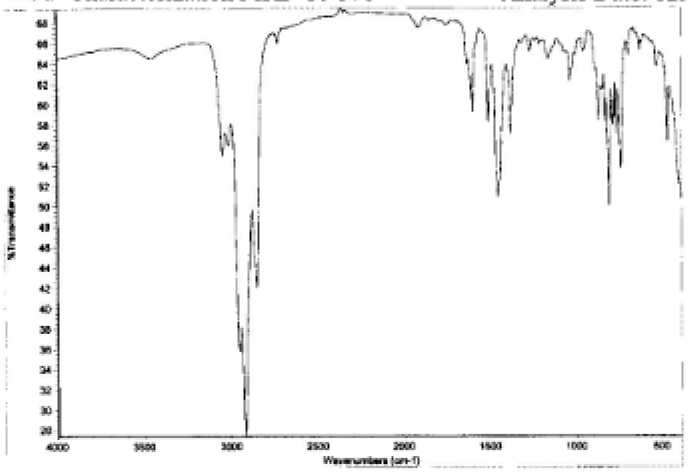
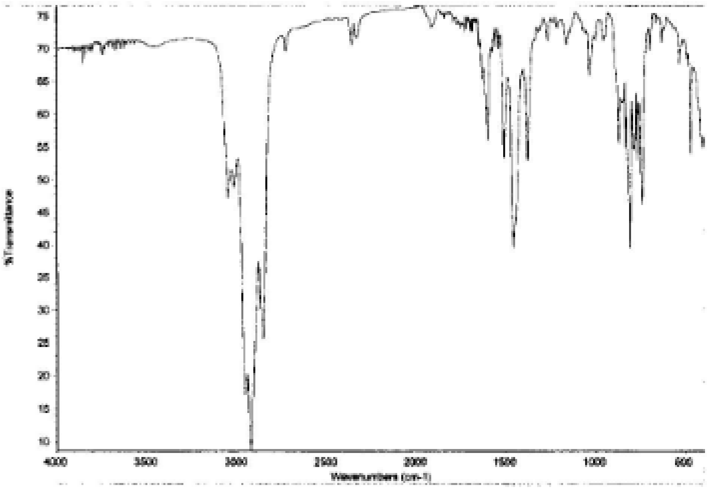


Figure: FTIR-2
Final

Final Characterization MRD-10-576 Analysis Date: 26Jul11



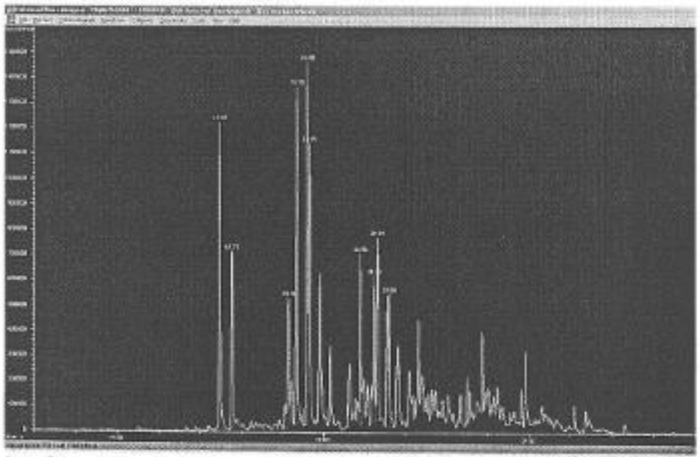
APPENDIX B – TEST SUBSTANCE CHARACTERIZATION (CONT'D)

TEST SUBSTANCE CHARACTERIZATION (CONT'D)

TOTAL ION CHROMATOGRAM

Figure: Total IonChromatogram-1

INITIAL



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Area Percent Report

  Data File : C:\HPCHEM\1\DATA\CHAR2010\12JUL02.D              Vial: 11
  Acq On    : 12 Jul 2010  23:03                             Operator:
  Sample    : MRD-10-576 (initial characterization) 10 Inst    : GC/MS Ins
  Misc      : distillates(petroleum)light catalytic cr Multiplr: 1.00
                                                    Sample Amount: 0.00
  MS Integration Params: MRD10576.E

  Method    : C:\HPCHEM\1\METHODS\CHAR2010.M (Chemstation Integrator)
  Title     :

  Signal    : TIC

 peak  R.T. first  max last  PK   peak      corr.    corr.    % of
   #   min  scan scan scan  TY  height     area    % max.   total
 ---  ----- ----- ---- ---- ---  -------  -------  ------  -------
   1  17.473  1827 1838 1855 BB   1191344  28114933  69.16%  13.215%
   2  17.769  1869 1875 1886 BB    696574  15482359  38.08%   7.277%
   3  19.147  2039 2047 2052 VV 2  507182  12837217  31.58%   6.034%
   4  19.348  2063 2072 2084 PV   1307221  38097107  93.71%  17.908%
   5  19.604  2094 2104 2107 BV   1434524  40652172 100.00%  19.109%

   6  19.652  2107 2110 2126 VB   1081233  23014351  56.61%  10.818%
   7  20.894  2261 2265 2270 VV    656953  14111799  34.71%   6.633%
   8  21.230  2303 2307 2313 VV    566030  13289573  32.69%   6.247%
   9  21.311  2313 2317 2329 VB    703810  14853731  36.54%   6.982%
  10  21.599  2351 2353 2368 VB    516463  12290409  30.23%   5.777%


                        Sum of corrected areas:    212743651

 12JUL02.D  CHAR2010.M      Wed Jul 14 08:12:47 2010
```

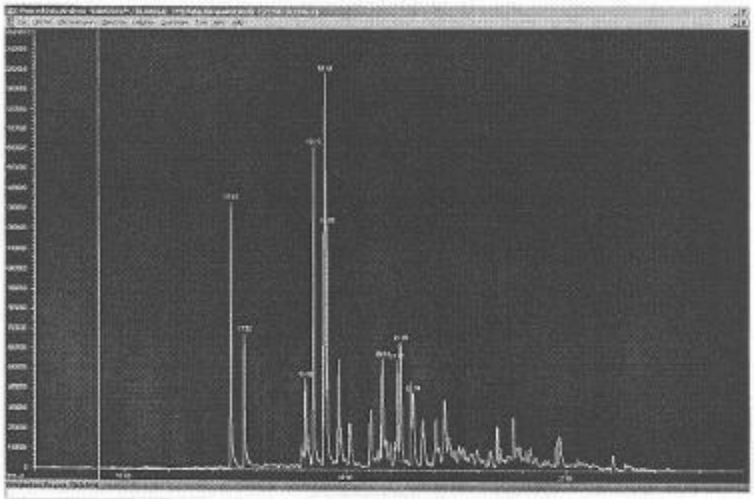
APPENDIX B – TEST SUBSTANCE CHARACTERIZATION (CONT'D)

TEST SUBSTANCE CHARACTERIZATION (CONT'D)

TOTAL ION CHROMATOGRAM

Figure: Total Ion Chromatogram-2

FINAL



```
Area Percent Report

  Data File : C:\HPCHEM\1\DATA\CHAR2010\26JUL02.D              Vial: 2
  Acq On    : 26 Jul 2011   9:52                            Operator:
  Sample    : MRD-10-576(final characterization)10%v/v Inst    : GC/MS Ins
  Misc      : distillates(petroleum)light catalytic cr Multiplr: 1.00
                                                     Sample Amount: 0.00
  MS Integration Params: 576.E

  Method    : C:\HPCHEM\1\METHODS\CHAR2010.M (Chemstation Integrator)
  Title     :

  Signal    : TIC

 peak  R.T. first  max last  PK   peak      corr.    corr.    % of
  #    min   scan scan scan  TY  height     area    % max.   total
 ---  ----- ----- ---- ---- ---  -------  -------   ------  -------
  1  17.400  2754 2767 2794 BB   1311520  26850418  62.74%  13.748%
  2  17.702  2816 2824 2842 BV    661262  13603665  31.79%   6.966%
  3  19.084  3079 3085 3092 VV 2  431463   8926702  20.86%   4.571%
  4  19.260  3111 3122 3141 PV   1601499  38831581  90.73%  19.883%
  5  19.529  3158 3169 3174 BV   1929536  42798102 100.00%  21.914%

  6  19.582  3174 3179 3207 VB   1191563  23621237  55.19%  12.095%
  7  20.631  3409 3415 3427 PV    476809   8948598  20.91%   4.582%
  8  21.170  3472 3479 3487 VV    505752  10273209  24.00%   5.260%
  9  21.250  3487 3494 3513 VB    586769  12203185  28.51%   6.248%
 10  21.536  3544 3548 3569 VB 3  357407   9243002  21.60%   4.733%


                        Sum of corrected areas:    195299499

  26JUL02.D  CHAR2010.M      Tue Jul 26 15:14:25 2011
```

APPENDIX C – SPONSOR SUPPLIED TEST SUBSTANCE INFORMATION

Intertek **Report of Analysis** **INTERIM**

[redacted]

[redacted]

Sample ID: 2009-DRPK-000651-012
Drawn By: Client
Sample Designated As: Crude Oil
Representing: Site#5 Sx.#1 (As Received)

Date Taken: 15-January-2009
Date Submitted: 15-January-2009
Date Tested: 26-January-2009

Method	Test	Result	Units
ASTM D4052	Density of Liquids by Digital Density Meter		
	Relative Density @ 60/60°F	0.9618	
	API Gravity @ 60°F	15.6	°API
ASTM D2887	Boiling Range Distribution of Petroleum Fractions by GC (Simulated Distillation)		
	Boiling Point Distribution	See Attached Report	
ASTM D1319	Hydrocarbon Types (Aromatics, Olefins, Saturates) by FIA		
	Aromatics	75.3	Vol %
	Olefins	7.2	Vol %
	Saturates	17.5	Vol %
ASTM D5186	Determination of Aromatic Content and Polynuclear Aromatic Content of Diesel Fuels and Aviation Turbine Fuels by Supercritical Fluid Chromatography		
	Monoaromatics by SFC	24.0	Wt %
	Polynuclear Aromatics by SFC	> 50.0	Wt %
	Total Aromatics	83.5	Wt %

[redacted]

[redacted]

[redacted]

[redacted]

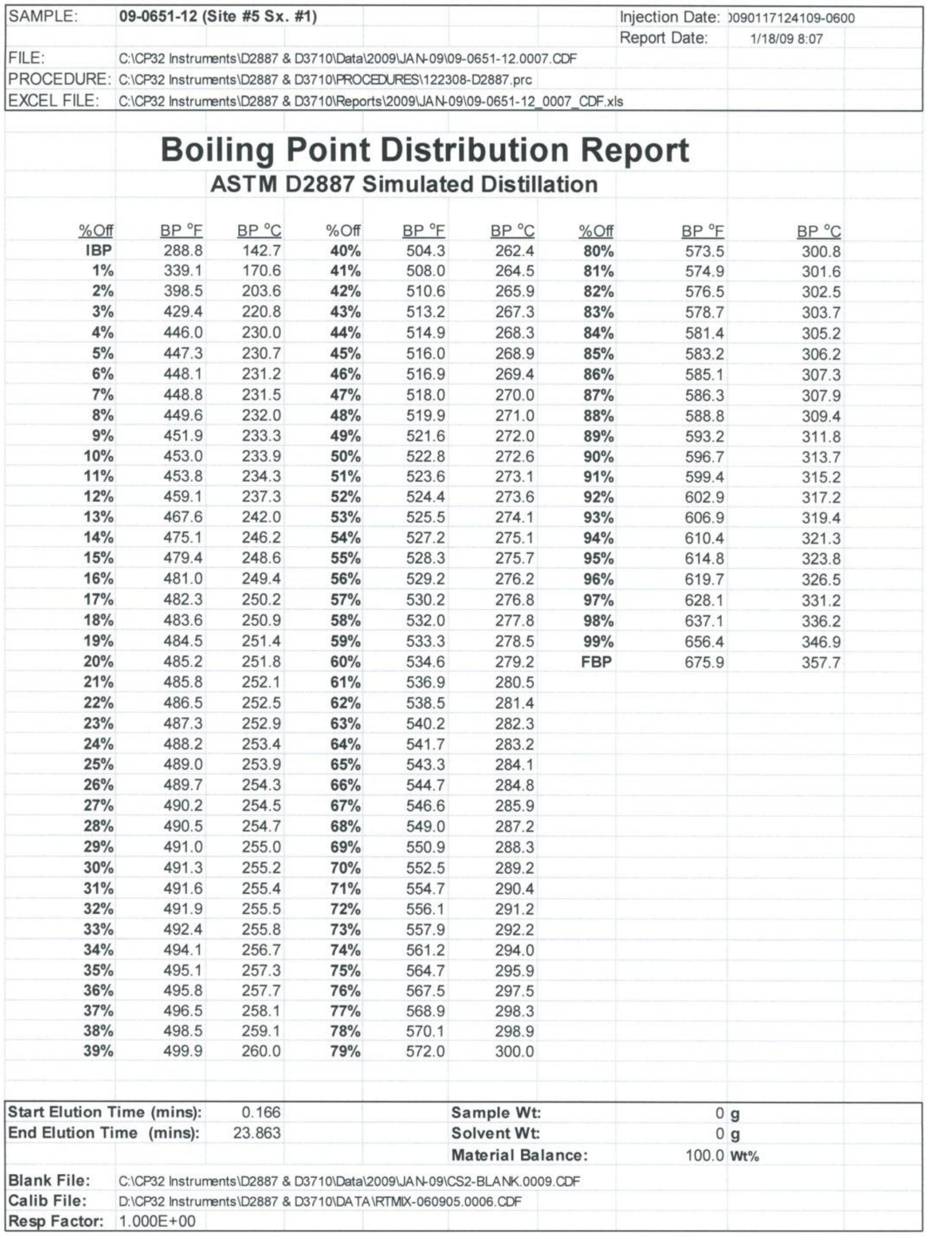
Page 4 of 16
61494

Information not relevant to the test sample has been blacked out.

1114 Seaco Avenue, Deer Park, Texas 77536 USA
Tel.: (713) 844-3200

05-Feb-2009 15:09
US785-0016408

APPENDIX C – SPONSOR SUPPLIED TEST SUBSTANCE INFORMATION (CONT'D)



SAMPLE: **09-0651-12 (Site #5 Sx. #1)**
Injection Date: 090117124109-0600
Report Date: 1/18/09 8:07
FILE: C:\CP32 Instruments\D2887 & D3710\Data\2009\JAN-09\09-0651-12.0007.CDF
PROCEDURE: C:\CP32 Instruments\D2887 & D3710\PROCEDURES\122308-D2887.prc
EXCEL FILE: C:\CP32 Instruments\D2887 & D3710\Reports\2009\JAN-09\09-0651-12_0007_CDF.xls

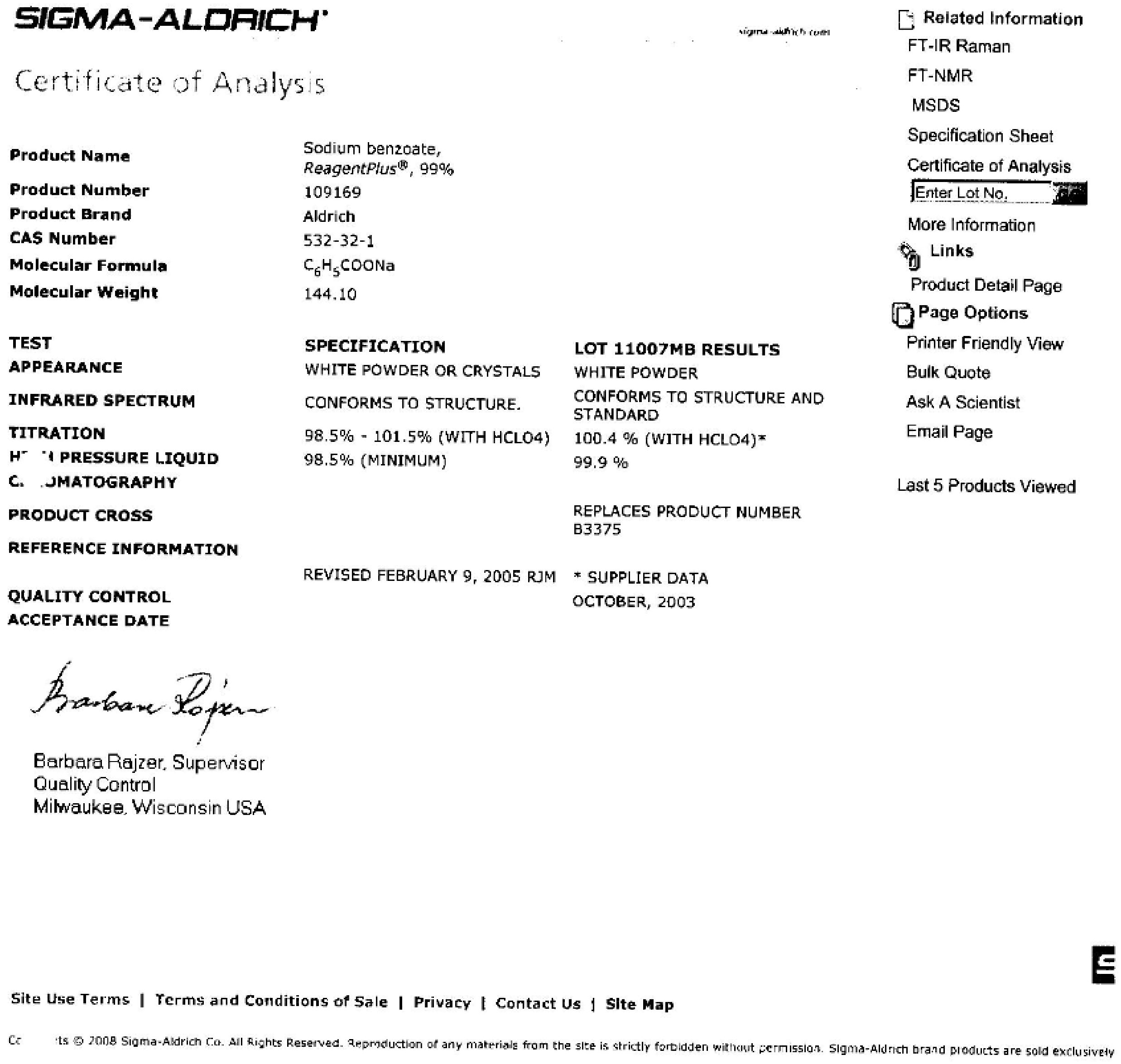
Boiling Point Distribution Report

ASTM D2887 Simulated Distillation

%Off	BP °F	BP °C	%Off	BP °F	BP °C	%Off	BP °F	BP °C
IBP	288.8	142.7	**40%**	504.3	262.4	**80%**	573.5	300.8
1%	339.1	170.6	**41%**	508.0	264.5	**81%**	574.9	301.6
2%	398.5	203.6	**42%**	510.6	265.9	**82%**	576.5	302.5
3%	429.4	220.8	**43%**	513.2	267.3	**83%**	578.7	303.7
4%	446.0	230.0	**44%**	514.9	268.3	**84%**	581.4	305.2
5%	447.3	230.7	**45%**	516.0	268.9	**85%**	583.2	306.2
6%	448.1	231.2	**46%**	516.9	269.4	**86%**	585.1	307.3
7%	448.8	231.5	**47%**	518.0	270.0	**87%**	586.3	307.9
8%	449.6	232.0	**48%**	519.9	271.0	**88%**	588.8	309.4
9%	451.9	233.3	**49%**	521.6	272.0	**89%**	593.2	311.8
10%	453.0	233.9	**50%**	522.8	272.6	**90%**	596.7	313.7
11%	453.8	234.3	**51%**	523.6	273.1	**91%**	599.4	315.2
12%	459.1	237.3	**52%**	524.4	273.6	**92%**	602.9	317.2
13%	467.6	242.0	**53%**	525.5	274.1	**93%**	606.9	319.4
14%	475.1	246.2	**54%**	527.2	275.1	**94%**	610.4	321.3
15%	479.4	248.6	**55%**	528.3	275.7	**95%**	614.8	323.8
16%	481.0	249.4	**56%**	529.2	276.2	**96%**	619.7	326.5
17%	482.3	250.2	**57%**	530.2	276.8	**97%**	628.1	331.2
18%	483.6	250.9	**58%**	532.0	277.8	**98%**	637.1	336.2
19%	484.5	251.4	**59%**	533.3	278.5	**99%**	656.4	346.9
20%	485.2	251.8	**60%**	534.6	279.2	**FBP**	675.9	357.7
21%	485.8	252.1	**61%**	536.9	280.5			
22%	486.5	252.5	**62%**	538.5	281.4			
23%	487.3	252.9	**63%**	540.2	282.3			
24%	488.2	253.4	**64%**	541.7	283.2			
25%	489.0	253.9	**65%**	543.3	284.1			
26%	489.7	254.3	**66%**	544.7	284.8			
27%	490.2	254.5	**67%**	546.6	285.9			
28%	490.5	254.7	**68%**	549.0	287.2			
29%	491.0	255.0	**69%**	550.9	288.3			
30%	491.3	255.2	**70%**	552.5	289.2			
31%	491.6	255.4	**71%**	554.7	290.4			
32%	491.9	255.5	**72%**	556.1	291.2			
33%	492.4	255.8	**73%**	557.9	292.2			
34%	494.1	256.7	**74%**	561.2	294.0			
35%	495.1	257.3	**75%**	564.7	295.9			
36%	495.8	257.7	**76%**	567.5	297.5			
37%	496.5	258.1	**77%**	568.9	298.3			
38%	498.5	259.1	**78%**	570.1	298.9			
39%	499.9	260.0	**79%**	572.0	300.0			

Start Elution Time (mins): 0.166
End Elution Time (mins): 23.863
Sample Wt: 0 **g**
Solvent Wt: 0 **g**
Material Balance: 100.0 **Wt%**
Blank File: C:\CP32 Instruments\D2887 & D3710\Data\2009\JAN-09\CS2-BLANK.0009.CDF
Calib File: D:\CP32 Instruments\D2887 & D3710\DATA\RTMIX-060905.0006.CDF
Resp Factor: 1.000E+00

APPENDIX D - SODIUM BENZOATE CERTIFICATE OF ANALYSIS



SIGMA-ALDRICH®

sigma-aldrich.com

Certificate of Analysis

Product Name	Sodium benzoate, *ReagentPlus®*, 99%
Product Number	109169
Product Brand	Aldrich
CAS Number	532-32-1
Molecular Formula	C_6H_5COONa
Molecular Weight	144.10

TEST	**SPECIFICATION**	**LOT 11007MB RESULTS**
APPEARANCE	WHITE POWDER OR CRYSTALS	WHITE POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.	CONFORMS TO STRUCTURE AND STANDARD
TITRATION	98.5% - 101.5% (WITH HCLO4)	100.4 % (WITH HCLO4)*
HIGH PRESSURE LIQUID CHROMATOGRAPHY	98.5% (MINIMUM)	99.9 %
PRODUCT CROSS REFERENCE INFORMATION		REPLACES PRODUCT NUMBER B3375
	REVISED FEBRUARY 9, 2005 RJM	* SUPPLIER DATA
QUALITY CONTROL ACCEPTANCE DATE		OCTOBER, 2003

Barbara Rajzer

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Related Information
FT-IR Raman
FT-NMR
MSDS
Specification Sheet
Certificate of Analysis
Enter Lot No.
More Information

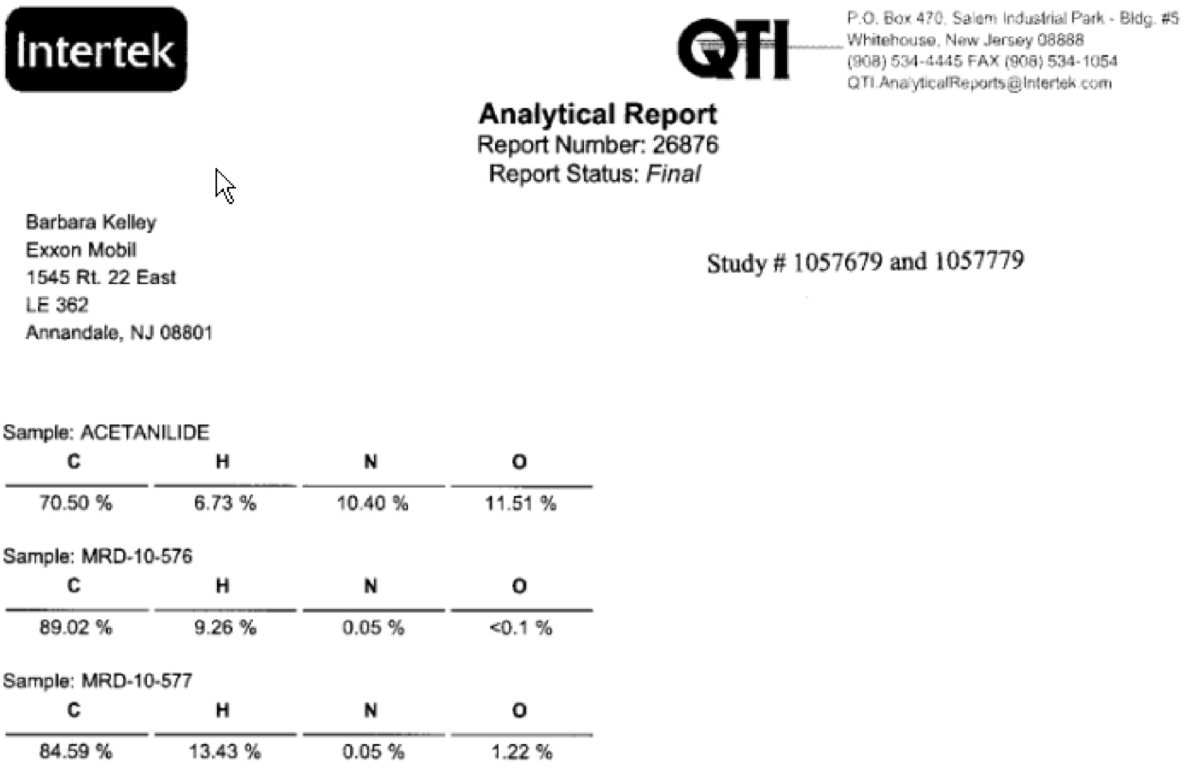
Links
Product Detail Page

Page Options
Printer Friendly View
Bulk Quote
Ask A Scientist
Email Page

Last 5 Products Viewed

Site Use Terms | Terms and Conditions of Sale | Privacy | Contact Us | Site Map

APPENDIX E – TEST SUBSTANCE ELEMENTAL ANALYSIS



Intertek

QTI
P.O. Box 470, Salem Industrial Park - Bldg. #5
Whitehouse, New Jersey 08888
(908) 534-4445 FAX (908) 534-1054
QTI.AnalyticalReports@Intertek.com

Analytical Report
Report Number: 26876
Report Status: *Final*

Barbara Kelley
Exxon Mobil
1545 Rt. 22 East
LE 362
Annandale, NJ 08801

Study # 1057679 and 1057779

Sample: ACETANILIDE

C	H	N	O
70.50 %	6.73 %	10.40 %	11.51 %

Sample: MRD-10-576

C	H	N	O
89.02 %	9.26 %	0.05 %	<0.1 %

Sample: MRD-10-577

C	H	N	O
84.59 %	13.43 %	0.05 %	1.22 %

APPENDIX F – PROTOCOL AND PROTOCOL REVISION

Study Title: READY BIODEGRADABILITY: OECD 301F
Manometric Respirometry Test

EMBSI Study Number: 1057679

Test Substance: MRD-10-576

Date: 18-Jun-10

Room Number: LE327

Proposed Key Dates:

Experimental Start Date	14-Jul-10
Experimental Termination Date	11-Aug-10
Draft Report Completion Date	9-Sep-10
Final Report Completion Date	22-Oct-10

Approved By:

[redacted]

28 June 10
Date

Study Director
ExxonMobil Biomedical Sciences, Inc.
1545 Route 22 East, P.O. Box 971
Annandale, New Jersey 08801-0971

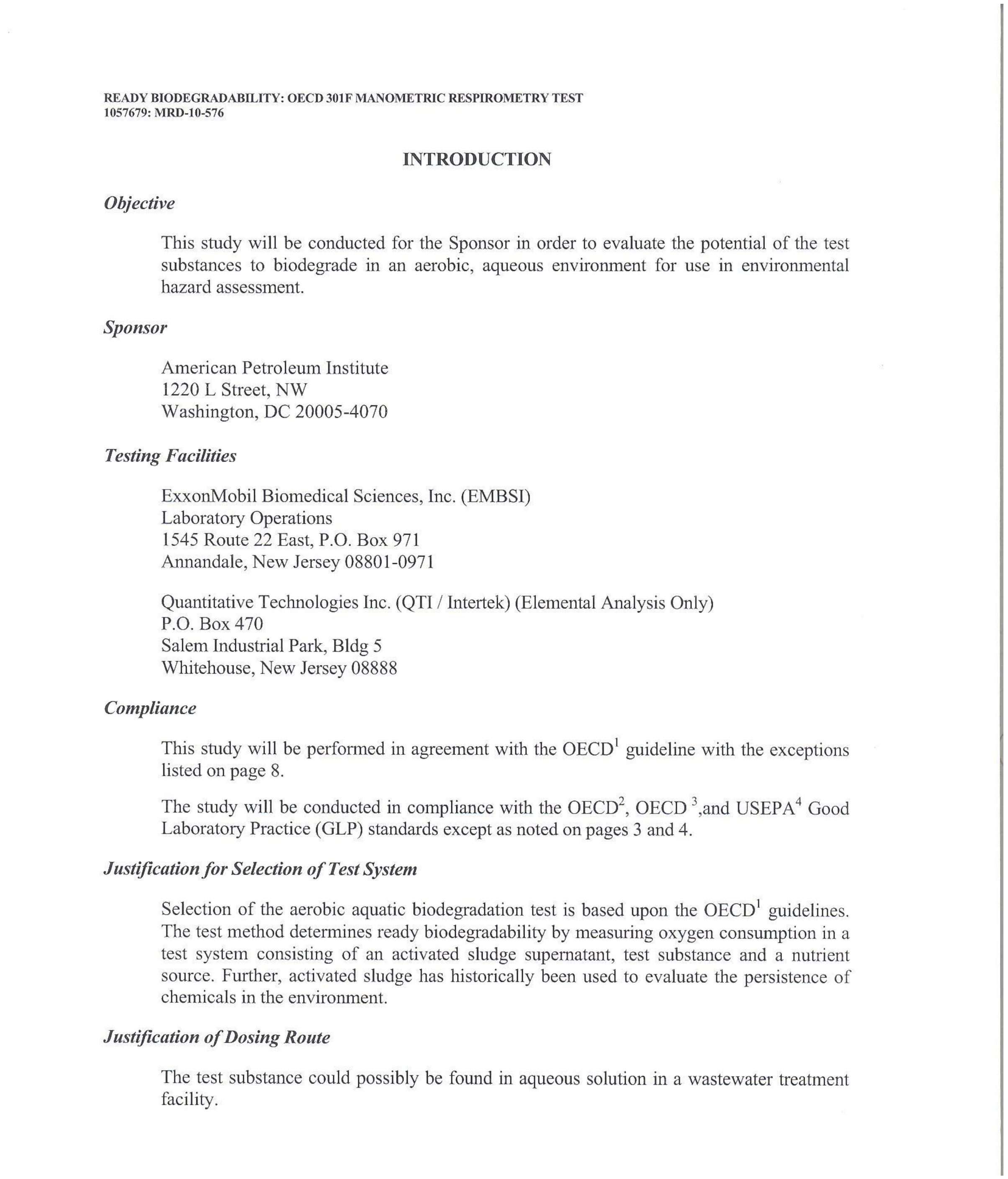
[redacted]

18 June 2010
Date

Sponsor Representative
American Petroleum Institute
Washington, DC

SAFETY FIRST

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT’D)



INTRODUCTION

Objective

This study will be conducted for the Sponsor in order to evaluate the potential of the test substances to biodegrade in an aerobic, aqueous environment for use in environmental hazard assessment.

Sponsor

American Petroleum Institute
1220 L Street, NW
Washington, DC 20005-4070

Testing Facilities

ExxonMobil Biomedical Sciences, Inc. (EMBSI)
Laboratory Operations
1545 Route 22 East, P.O. Box 971
Annandale, New Jersey 08801-0971

Quantitative Technologies Inc. (QTI / Intertek) (Elemental Analysis Only)
P.O. Box 470
Salem Industrial Park, Bldg 5
Whitehouse, New Jersey 08888

Compliance

This study will be performed in agreement with the OECD[1] guideline with the exceptions listed on page 8.

The study will be conducted in compliance with the OECD[2], OECD [3],and USEPA[4] Good Laboratory Practice (GLP) standards except as noted on pages 3 and 4.

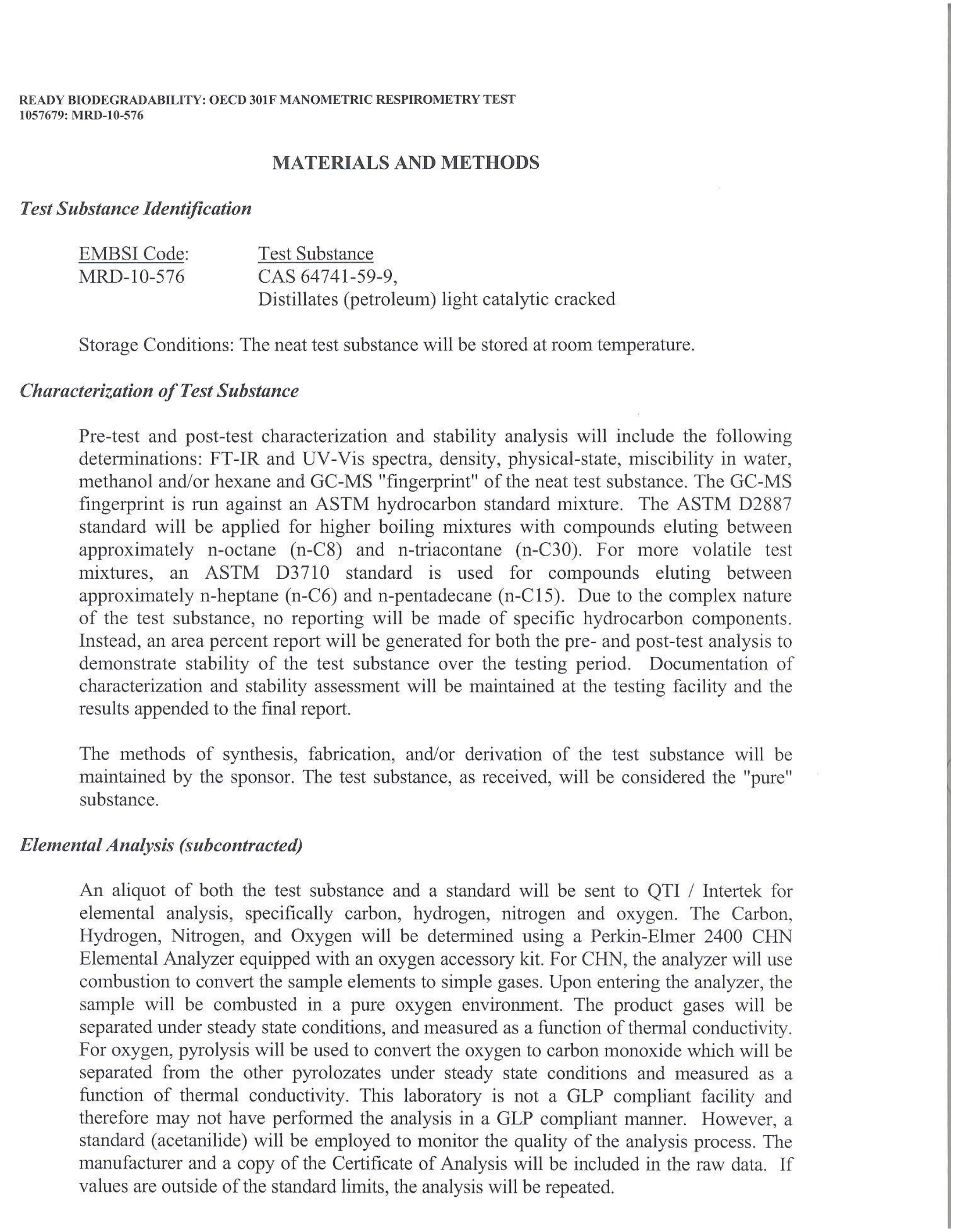
Justification for Selection of Test System

Selection of the aerobic aquatic biodegradation test is based upon the OECD[1] guidelines. The test method determines ready biodegradability by measuring oxygen consumption in a test system consisting of an activated sludge supernatant, test substance and a nutrient source. Further, activated sludge has historically been used to evaluate the persistence of chemicals in the environment.

Justification of Dosing Route

The test substance could possibly be found in aqueous solution in a wastewater treatment facility.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



MATERIALS AND METHODS

Test Substance Identification

EMBSI Code:	Test Substance
MRD-10-576	CAS 64741-59-9, Distillates (petroleum) light catalytic cracked

Storage Conditions: The neat test substance will be stored at room temperature.

Characterization of Test Substance

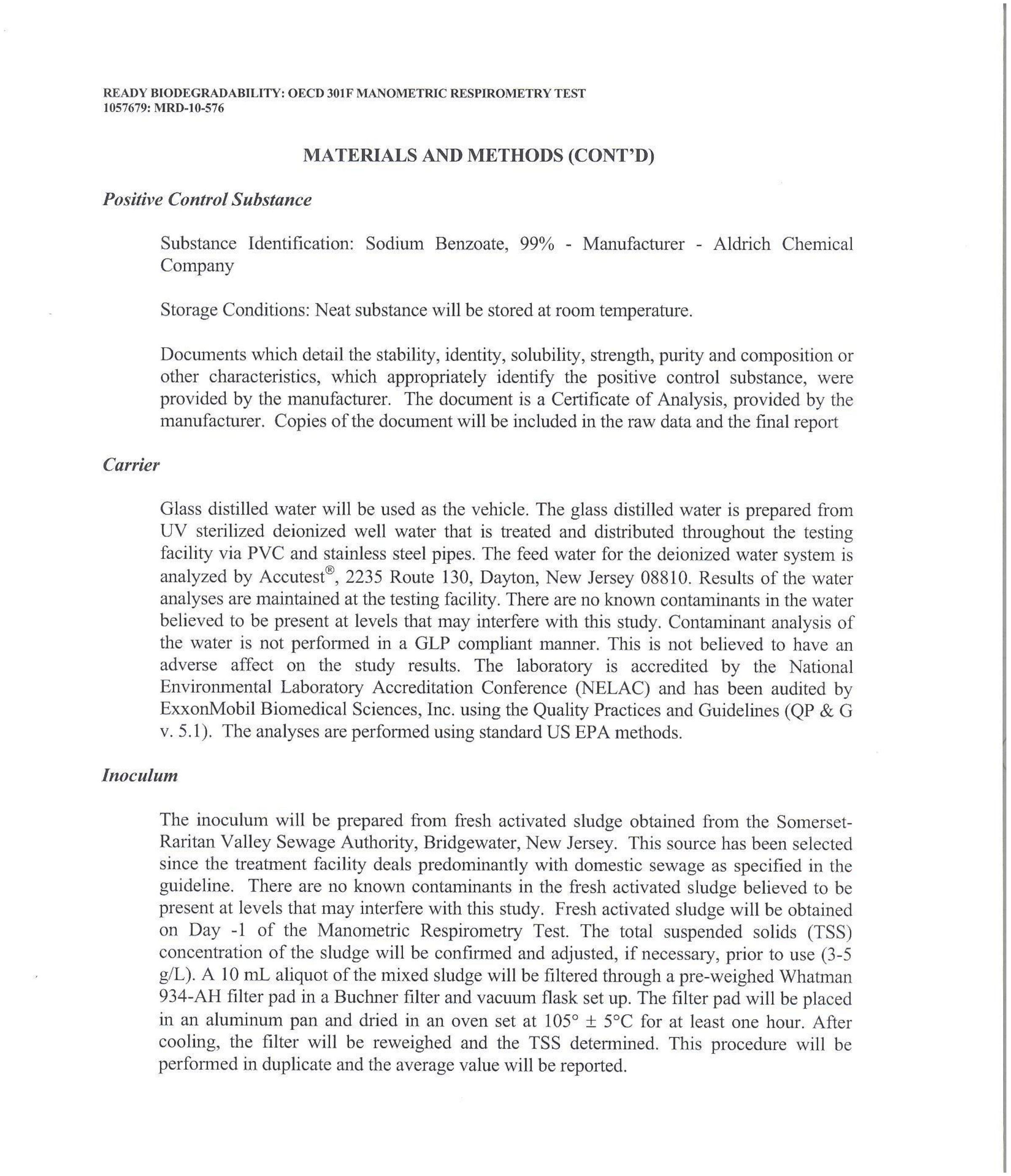
Pre-test and post-test characterization and stability analysis will include the following determinations: FT-IR and UV-Vis spectra, density, physical-state, miscibility in water, methanol and/or hexane and GC-MS "fingerprint" of the neat test substance. The GC-MS fingerprint is run against an ASTM hydrocarbon standard mixture. The ASTM D2887 standard will be applied for higher boiling mixtures with compounds eluting between approximately n-octane (n-C8) and n-triacontane (n-C30). For more volatile test mixtures, an ASTM D3710 standard is used for compounds eluting between approximately n-heptane (n-C6) and n-pentadecane (n-C15). Due to the complex nature of the test substance, no reporting will be made of specific hydrocarbon components. Instead, an area percent report will be generated for both the pre- and post-test analysis to demonstrate stability of the test substance over the testing period. Documentation of characterization and stability assessment will be maintained at the testing facility and the results appended to the final report.

The methods of synthesis, fabrication, and/or derivation of the test substance will be maintained by the sponsor. The test substance, as received, will be considered the "pure" substance.

Elemental Analysis (subcontracted)

An aliquot of both the test substance and a standard will be sent to QTI / Intertek for elemental analysis, specifically carbon, hydrogen, nitrogen and oxygen. The Carbon, Hydrogen, Nitrogen, and Oxygen will be determined using a Perkin-Elmer 2400 CHN Elemental Analyzer equipped with an oxygen accessory kit. For CHN, the analyzer will use combustion to convert the sample elements to simple gases. Upon entering the analyzer, the sample will be combusted in a pure oxygen environment. The product gases will be separated under steady state conditions, and measured as a function of thermal conductivity. For oxygen, pyrolysis will be used to convert the oxygen to carbon monoxide which will be separated from the other pyrolozates under steady state conditions and measured as a function of thermal conductivity. This laboratory is not a GLP compliant facility and therefore may not have performed the analysis in a GLP compliant manner. However, a standard (acetanilide) will be employed to monitor the quality of the analysis process. The manufacturer and a copy of the Certificate of Analysis will be included in the raw data. If values are outside of the standard limits, the analysis will be repeated.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



MATERIALS AND METHODS (CONT'D)

Positive Control Substance

Substance Identification: Sodium Benzoate, 99% - Manufacturer - Aldrich Chemical Company

Storage Conditions: Neat substance will be stored at room temperature.

Documents which detail the stability, identity, solubility, strength, purity and composition or other characteristics, which appropriately identify the positive control substance, were provided by the manufacturer. The document is a Certificate of Analysis, provided by the manufacturer. Copies of the document will be included in the raw data and the final report

Carrier

Glass distilled water will be used as the vehicle. The glass distilled water is prepared from UV sterilized deionized well water that is treated and distributed throughout the testing facility via PVC and stainless steel pipes. The feed water for the deionized water system is analyzed by Accutest®, 2235 Route 130, Dayton, New Jersey 08810. Results of the water analyses are maintained at the testing facility. There are no known contaminants in the water believed to be present at levels that may interfere with this study. Contaminant analysis of the water is not performed in a GLP compliant manner. This is not believed to have an adverse affect on the study results. The laboratory is accredited by the National Environmental Laboratory Accreditation Conference (NELAC) and has been audited by ExxonMobil Biomedical Sciences, Inc. using the Quality Practices and Guidelines (QP & G v. 5.1). The analyses are performed using standard US EPA methods.

Inoculum

The inoculum will be prepared from fresh activated sludge obtained from the Somerset-Raritan Valley Sewage Authority, Bridgewater, New Jersey. This source has been selected since the treatment facility deals predominantly with domestic sewage as specified in the guideline. There are no known contaminants in the fresh activated sludge believed to be present at levels that may interfere with this study. Fresh activated sludge will be obtained on Day -1 of the Manometric Respirometry Test. The total suspended solids (TSS) concentration of the sludge will be confirmed and adjusted, if necessary, prior to use (3-5 g/L). A 10 mL aliquot of the mixed sludge will be filtered through a pre-weighed Whatman 934-AH filter pad in a Buchner filter and vacuum flask set up. The filter pad will be placed in an aluminum pan and dried in an oven set at 105° ± 5°C for at least one hour. After cooling, the filter will be reweighed and the TSS determined. This procedure will be performed in duplicate and the average value will be reported.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)

MATERIALS AND METHODS (CONT'D)

Inoculum (Cont'd)

The sludge will be homogenized for 2 minutes in a blender at medium speed. The homogenized sample will be allowed to settle for at least 30 minutes, after which the supernatant will be decanted (avoiding carry-over of sludge solids). Settling time will be dictated by the clarity of the supernatant. An aliquot of the supernatant will be used for final preparation of the test medium (see test medium preparation). An aliquot of the remaining supernatant will be used to determine microbial activity with an Easicult®-TTC dipslide. This will be accomplished by removing the agar stick from the culturing tube, and dipping the agar into the supernatant aliquot. Excess supernatant will be blotted off with a clean paper towel, and the agar stick will then be placed back into the culture tube. The whole unit will be placed into a dark Environmental Chamber for 48 hours at 20 ± 1°C. After 48 hours, the whole unit will be observed, and the density of the colonies growing on the medium will be compared to the model density chart provided by the supplier. The density will be reported as CFU/mL, (colony forming units per ml of sample) which is the microbial concentration.

Solutions

Mineral Salt Solutions: Phosphate buffer pH 7.2
Ferric chloride (0.025%)
Magnesium sulfate (2.25%)
Calcium chloride (2.75%)

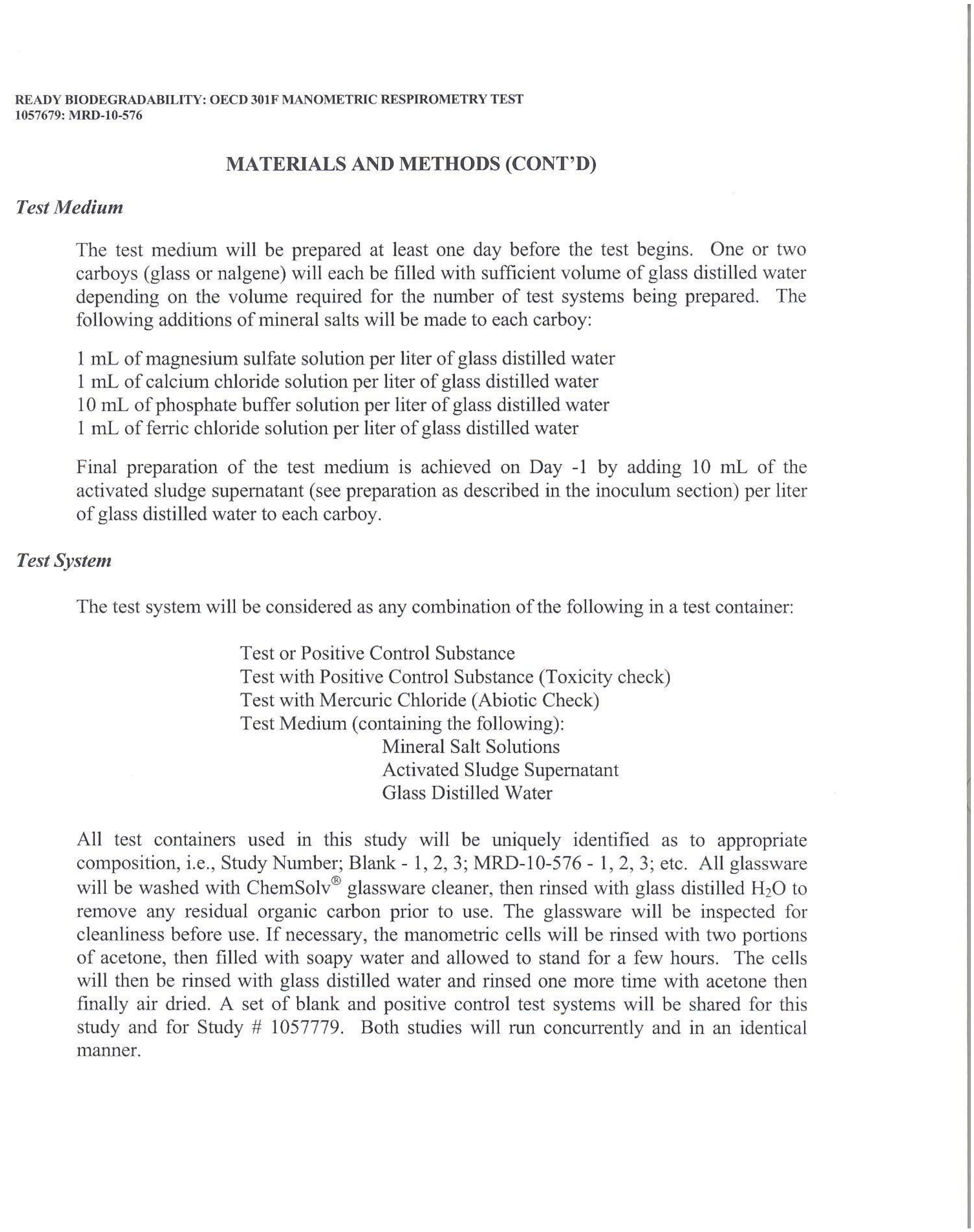
The manufacturer and lot number for each solution will be recorded in both the raw data and the report. There are no known contaminants in the solutions believed to be present at levels that may interfere with this study. All solutions will be refrigerated when not in use.

Sodium Benzoate Stock Solution: A stock solution of sodium benzoate will be prepared in glass distilled water. The stock solution will be refrigerated when not in use.

The stock solution concentration will be verified by Total Organic Carbon (TOC) analysis as per SOP G.02.09.07.

The pH of the Sodium Benzoate stock will be measured, and adjusted to 7.4± 0.2 if necessary.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



MATERIALS AND METHODS (CONT'D)

Test Medium

The test medium will be prepared at least one day before the test begins. One or two carboys (glass or nalgene) will each be filled with sufficient volume of glass distilled water depending on the volume required for the number of test systems being prepared. The following additions of mineral salts will be made to each carboy:

1 mL of magnesium sulfate solution per liter of glass distilled water
1 mL of calcium chloride solution per liter of glass distilled water
10 mL of phosphate buffer solution per liter of glass distilled water
1 mL of ferric chloride solution per liter of glass distilled water

Final preparation of the test medium is achieved on Day -1 by adding 10 mL of the activated sludge supernatant (see preparation as described in the inoculum section) per liter of glass distilled water to each carboy.

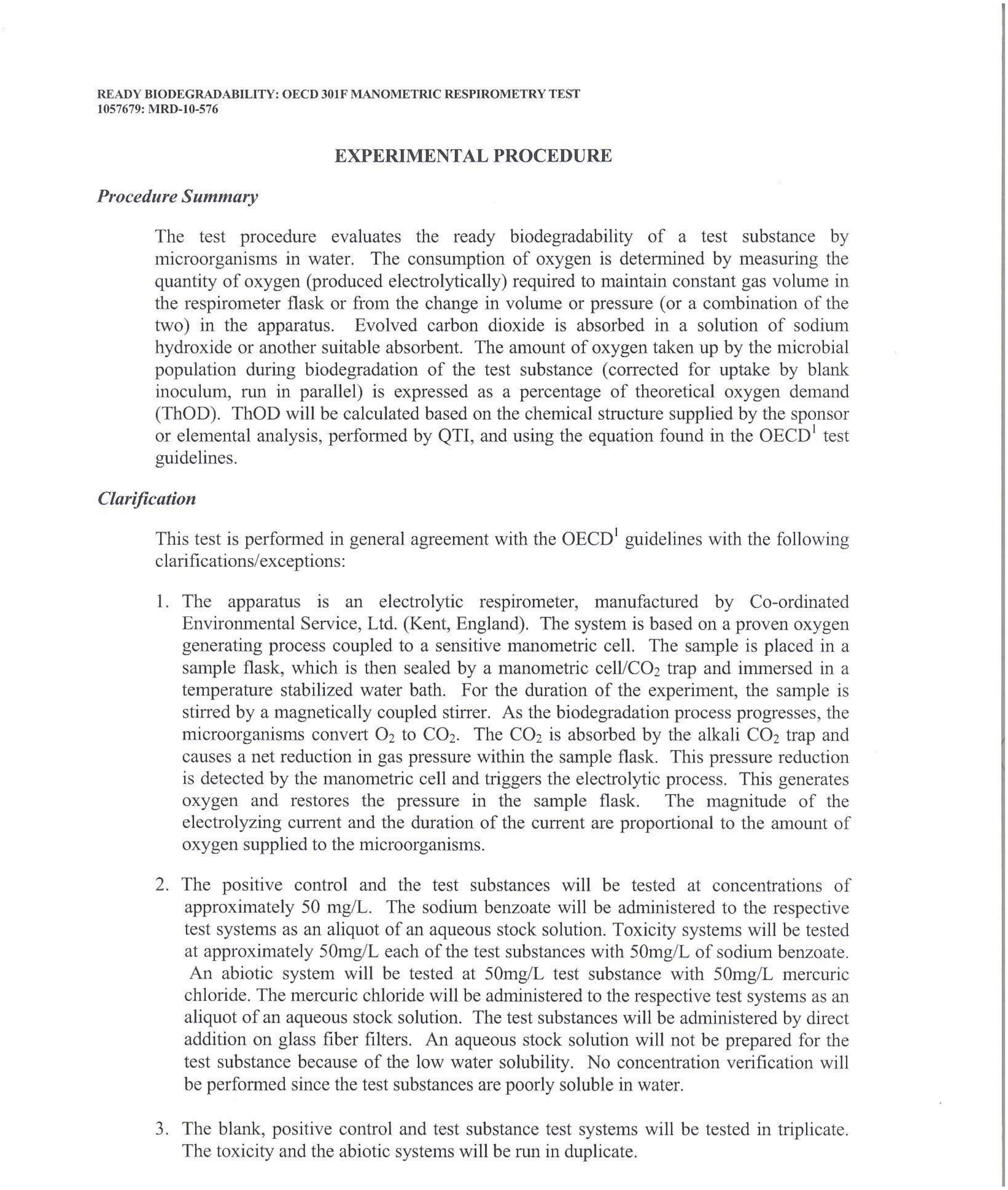
Test System

The test system will be considered as any combination of the following in a test container:

- Test or Positive Control Substance
- Test with Positive Control Substance (Toxicity check)
- Test with Mercuric Chloride (Abiotic Check)
- Test Medium (containing the following):
  - Mineral Salt Solutions
  - Activated Sludge Supernatant
  - Glass Distilled Water

All test containers used in this study will be uniquely identified as to appropriate composition, i.e., Study Number; Blank - 1, 2, 3; MRD-10-576 - 1, 2, 3; etc. All glassware will be washed with ChemSolv® glassware cleaner, then rinsed with glass distilled H_2O to remove any residual organic carbon prior to use. The glassware will be inspected for cleanliness before use. If necessary, the manometric cells will be rinsed with two portions of acetone, then filled with soapy water and allowed to stand for a few hours. The cells will then be rinsed with glass distilled water and rinsed one more time with acetone then finally air dried. A set of blank and positive control test systems will be shared for this study and for Study # 1057779. Both studies will run concurrently and in an identical manner.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



EXPERIMENTAL PROCEDURE

Procedure Summary

The test procedure evaluates the ready biodegradability of a test substance by microorganisms in water. The consumption of oxygen is determined by measuring the quantity of oxygen (produced electrolytically) required to maintain constant gas volume in the respirometer flask or from the change in volume or pressure (or a combination of the two) in the apparatus. Evolved carbon dioxide is absorbed in a solution of sodium hydroxide or another suitable absorbent. The amount of oxygen taken up by the microbial population during biodegradation of the test substance (corrected for uptake by blank inoculum, run in parallel) is expressed as a percentage of theoretical oxygen demand (ThOD). ThOD will be calculated based on the chemical structure supplied by the sponsor or elemental analysis, performed by QTI, and using the equation found in the OECD[1] test guidelines.

Clarification

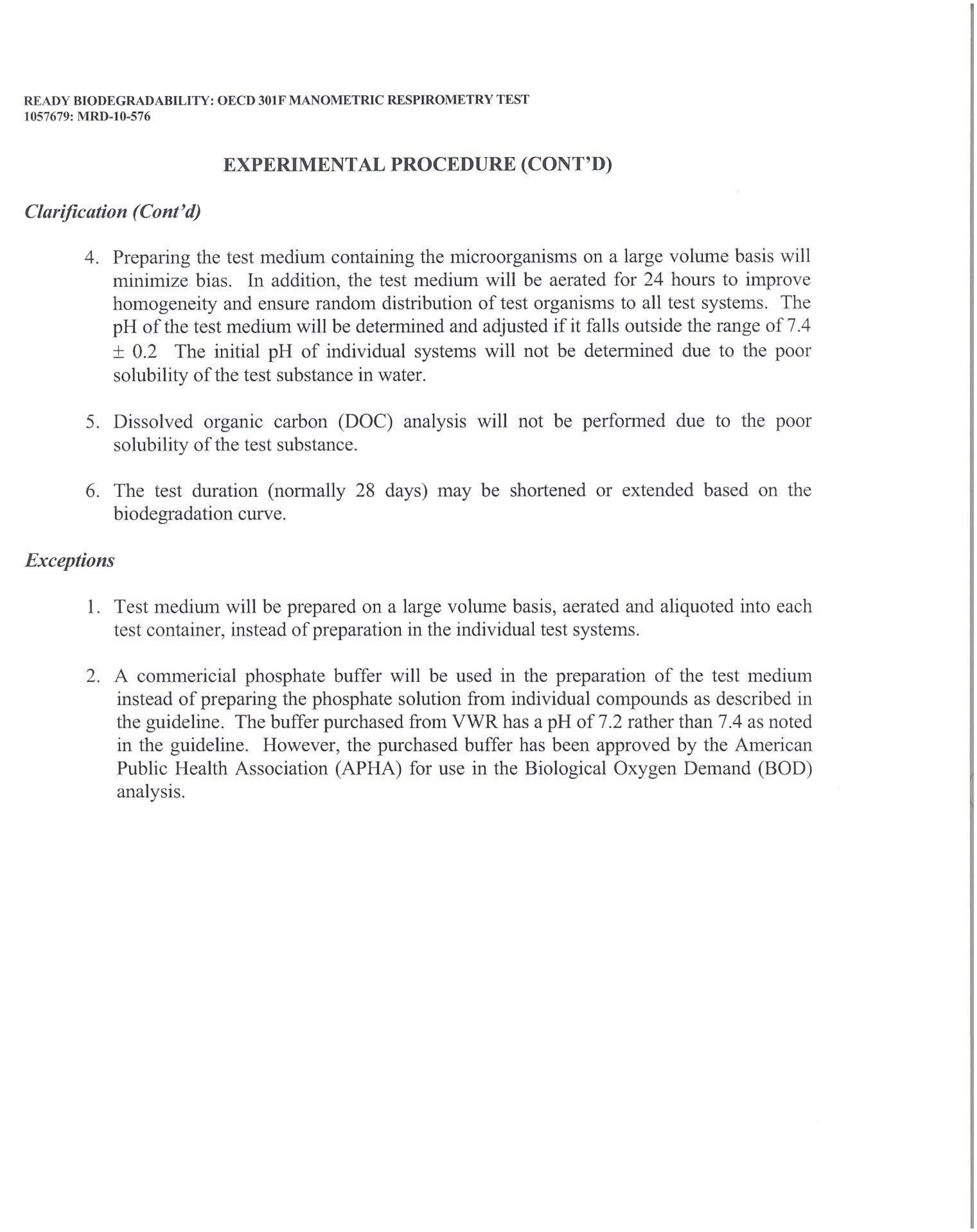
This test is performed in general agreement with the OECD[1] guidelines with the following clarifications/exceptions:

1. The apparatus is an electrolytic respirometer, manufactured by Co-ordinated Environmental Service, Ltd. (Kent, England). The system is based on a proven oxygen generating process coupled to a sensitive manometric cell. The sample is placed in a sample flask, which is then sealed by a manometric cell/CO_2 trap and immersed in a temperature stabilized water bath. For the duration of the experiment, the sample is stirred by a magnetically coupled stirrer. As the biodegradation process progresses, the microorganisms convert O_2 to CO_2. The CO_2 is absorbed by the alkali CO_2 trap and causes a net reduction in gas pressure within the sample flask. This pressure reduction is detected by the manometric cell and triggers the electrolytic process. This generates oxygen and restores the pressure in the sample flask. The magnitude of the electrolyzing current and the duration of the current are proportional to the amount of oxygen supplied to the microorganisms.

2. The positive control and the test substances will be tested at concentrations of approximately 50 mg/L. The sodium benzoate will be administered to the respective test systems as an aliquot of an aqueous stock solution. Toxicity systems will be tested at approximately 50mg/L each of the test substances with 50mg/L of sodium benzoate. An abiotic system will be tested at 50mg/L test substance with 50mg/L mercuric chloride. The mercuric chloride will be administered to the respective test systems as an aliquot of an aqueous stock solution. The test substances will be administered by direct addition on glass fiber filters. An aqueous stock solution will not be prepared for the test substance because of the low water solubility. No concentration verification will be performed since the test substances are poorly soluble in water.

3. The blank, positive control and test substance test systems will be tested in triplicate. The toxicity and the abiotic systems will be run in duplicate.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



EXPERIMENTAL PROCEDURE (CONT'D)

Clarification (Cont'd)

4. Preparing the test medium containing the microorganisms on a large volume basis will minimize bias. In addition, the test medium will be aerated for 24 hours to improve homogeneity and ensure random distribution of test organisms to all test systems. The pH of the test medium will be determined and adjusted if it falls outside the range of 7.4 ± 0.2 The initial pH of individual systems will not be determined due to the poor solubility of the test substance in water.

5. Dissolved organic carbon (DOC) analysis will not be performed due to the poor solubility of the test substance.

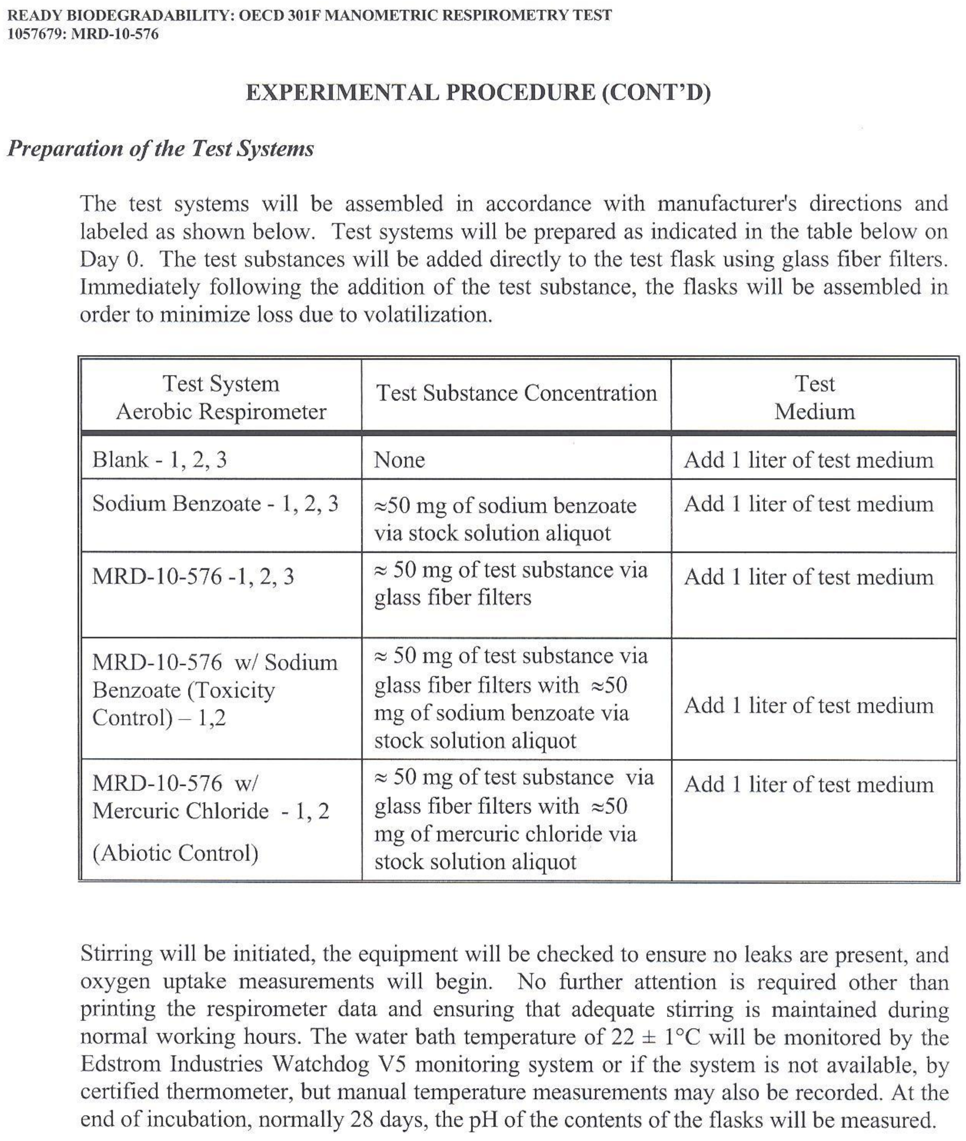
6. The test duration (normally 28 days) may be shortened or extended based on the biodegradation curve.

Exceptions

1. Test medium will be prepared on a large volume basis, aerated and aliquoted into each test container, instead of preparation in the individual test systems.

2. A commericial phosphate buffer will be used in the preparation of the test medium instead of preparing the phosphate solution from individual compounds as described in the guideline. The buffer purchased from VWR has a pH of 7.2 rather than 7.4 as noted in the guideline. However, the purchased buffer has been approved by the American Public Health Association (APHA) for use in the Biological Oxygen Demand (BOD) analysis.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



EXPERIMENTAL PROCEDURE (CONT'D)

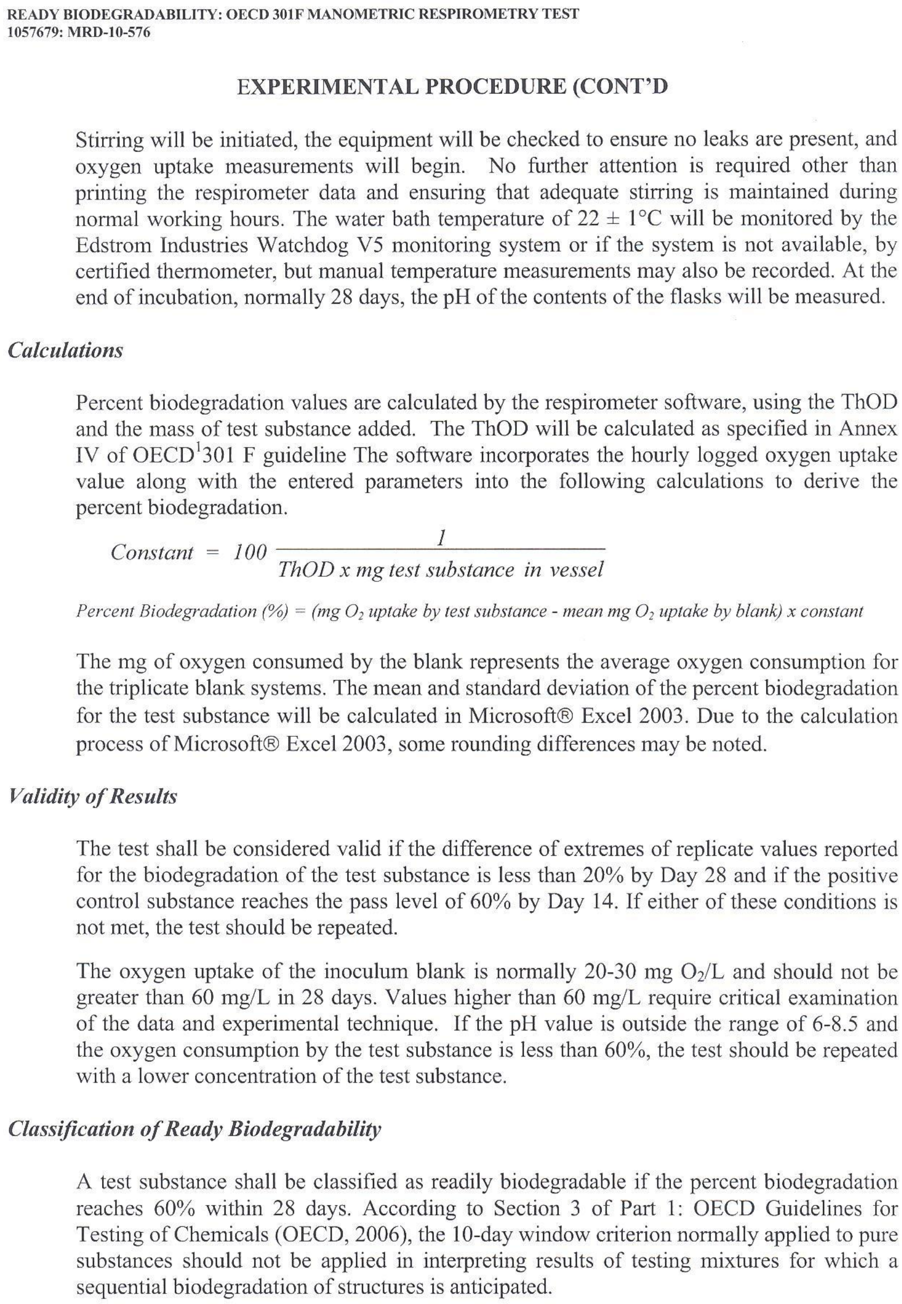
Preparation of the Test Systems

The test systems will be assembled in accordance with manufacturer's directions and labeled as shown below. Test systems will be prepared as indicated in the table below on Day 0. The test substances will be added directly to the test flask using glass fiber filters. Immediately following the addition of the test substance, the flasks will be assembled in order to minimize loss due to volatilization.

Test System Aerobic Respirometer	Test Substance Concentration	Test Medium
Blank - 1, 2, 3	None	Add 1 liter of test medium
Sodium Benzoate - 1, 2, 3	≈50 mg of sodium benzoate via stock solution aliquot	Add 1 liter of test medium
MRD-10-576 -1, 2, 3	≈ 50 mg of test substance via glass fiber filters	Add 1 liter of test medium
MRD-10-576 w/ Sodium Benzoate (Toxicity Control) – 1,2	≈ 50 mg of test substance via glass fiber filters with ≈50 mg of sodium benzoate via stock solution aliquot	Add 1 liter of test medium
MRD-10-576 w/ Mercuric Chloride - 1, 2 (Abiotic Control)	≈ 50 mg of test substance via glass fiber filters with ≈50 mg of mercuric chloride via stock solution aliquot	Add 1 liter of test medium

Stirring will be initiated, the equipment will be checked to ensure no leaks are present, and oxygen uptake measurements will begin. No further attention is required other than printing the respirometer data and ensuring that adequate stirring is maintained during normal working hours. The water bath temperature of 22 ± 1°C will be monitored by the Edstrom Industries Watchdog V5 monitoring system or if the system is not available, by certified thermometer, but manual temperature measurements may also be recorded. At the end of incubation, normally 28 days, the pH of the contents of the flasks will be measured.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



EXPERIMENTAL PROCEDURE (CONT'D

Stirring will be initiated, the equipment will be checked to ensure no leaks are present, and oxygen uptake measurements will begin. No further attention is required other than printing the respirometer data and ensuring that adequate stirring is maintained during normal working hours. The water bath temperature of 22 ± 1°C will be monitored by the Edstrom Industries Watchdog V5 monitoring system or if the system is not available, by certified thermometer, but manual temperature measurements may also be recorded. At the end of incubation, normally 28 days, the pH of the contents of the flasks will be measured.

Calculations

Percent biodegradation values are calculated by the respirometer software, using the ThOD and the mass of test substance added. The ThOD will be calculated as specified in Annex IV of OECD[1]301 F guideline The software incorporates the hourly logged oxygen uptake value along with the entered parameters into the following calculations to derive the percent biodegradation.

$$Constant = 100 \frac{1}{ThOD \times mg\ test\ substance\ in\ vessel}$$

$$Percent\ Biodegradation\ (\%) = (mg\ O_2\ uptake\ by\ test\ substance - mean\ mg\ O_2\ uptake\ by\ blank) \times constant$$

The mg of oxygen consumed by the blank represents the average oxygen consumption for the triplicate blank systems. The mean and standard deviation of the percent biodegradation for the test substance will be calculated in Microsoft® Excel 2003. Due to the calculation process of Microsoft® Excel 2003, some rounding differences may be noted.

Validity of Results

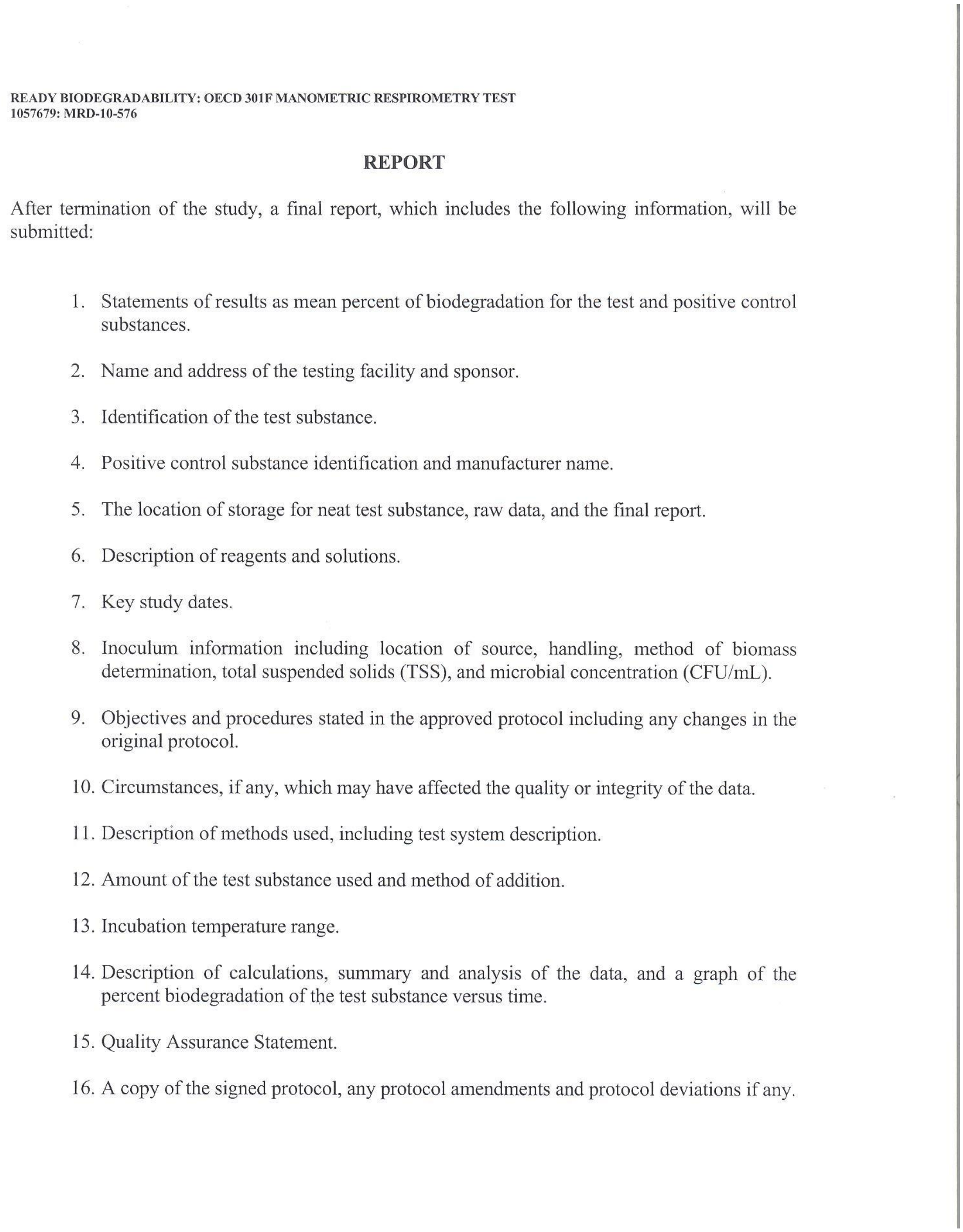
The test shall be considered valid if the difference of extremes of replicate values reported for the biodegradation of the test substance is less than 20% by Day 28 and if the positive control substance reaches the pass level of 60% by Day 14. If either of these conditions is not met, the test should be repeated.

The oxygen uptake of the inoculum blank is normally 20-30 mg O_2/L and should not be greater than 60 mg/L in 28 days. Values higher than 60 mg/L require critical examination of the data and experimental technique. If the pH value is outside the range of 6-8.5 and the oxygen consumption by the test substance is less than 60%, the test should be repeated with a lower concentration of the test substance.

Classification of Ready Biodegradability

A test substance shall be classified as readily biodegradable if the percent biodegradation reaches 60% within 28 days. According to Section 3 of Part 1: OECD Guidelines for Testing of Chemicals (OECD, 2006), the 10-day window criterion normally applied to pure substances should not be applied in interpreting results of testing mixtures for which a sequential biodegradation of structures is anticipated.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)

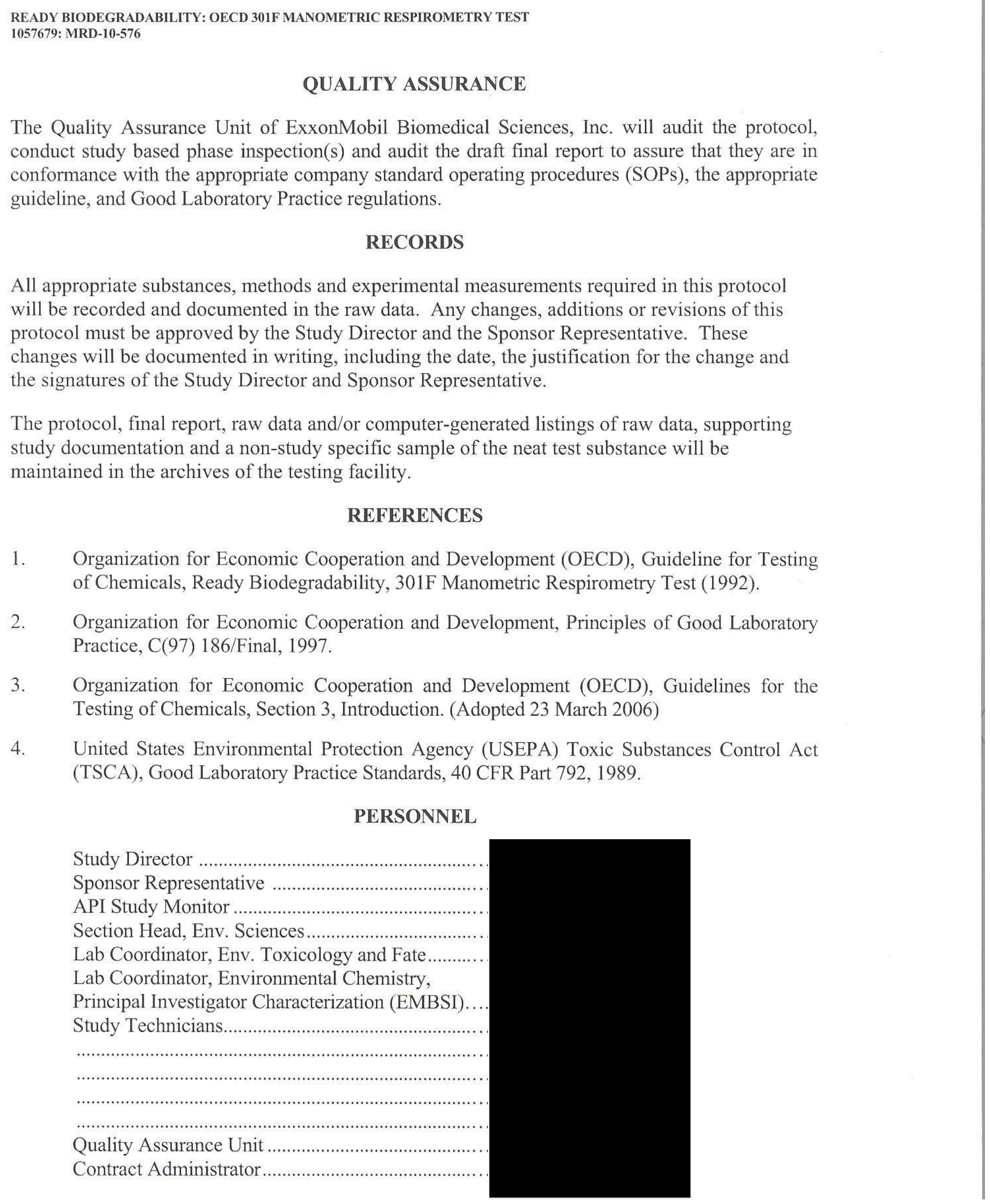


REPORT

After termination of the study, a final report, which includes the following information, will be submitted:

1. Statements of results as mean percent of biodegradation for the test and positive control substances.
2. Name and address of the testing facility and sponsor.
3. Identification of the test substance.
4. Positive control substance identification and manufacturer name.
5. The location of storage for neat test substance, raw data, and the final report.
6. Description of reagents and solutions.
7. Key study dates.
8. Inoculum information including location of source, handling, method of biomass determination, total suspended solids (TSS), and microbial concentration (CFU/mL).
9. Objectives and procedures stated in the approved protocol including any changes in the original protocol.
10. Circumstances, if any, which may have affected the quality or integrity of the data.
11. Description of methods used, including test system description.
12. Amount of the test substance used and method of addition.
13. Incubation temperature range.
14. Description of calculations, summary and analysis of the data, and a graph of the percent biodegradation of the test substance versus time.
15. Quality Assurance Statement.
16. A copy of the signed protocol, any protocol amendments and protocol deviations if any.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



QUALITY ASSURANCE

The Quality Assurance Unit of ExxonMobil Biomedical Sciences, Inc. will audit the protocol, conduct study based phase inspection(s) and audit the draft final report to assure that they are in conformance with the appropriate company standard operating procedures (SOPs), the appropriate guideline, and Good Laboratory Practice regulations.

RECORDS

All appropriate substances, methods and experimental measurements required in this protocol will be recorded and documented in the raw data. Any changes, additions or revisions of this protocol must be approved by the Study Director and the Sponsor Representative. These changes will be documented in writing, including the date, the justification for the change and the signatures of the Study Director and Sponsor Representative.

The protocol, final report, raw data and/or computer-generated listings of raw data, supporting study documentation and a non-study specific sample of the neat test substance will be maintained in the archives of the testing facility.

REFERENCES

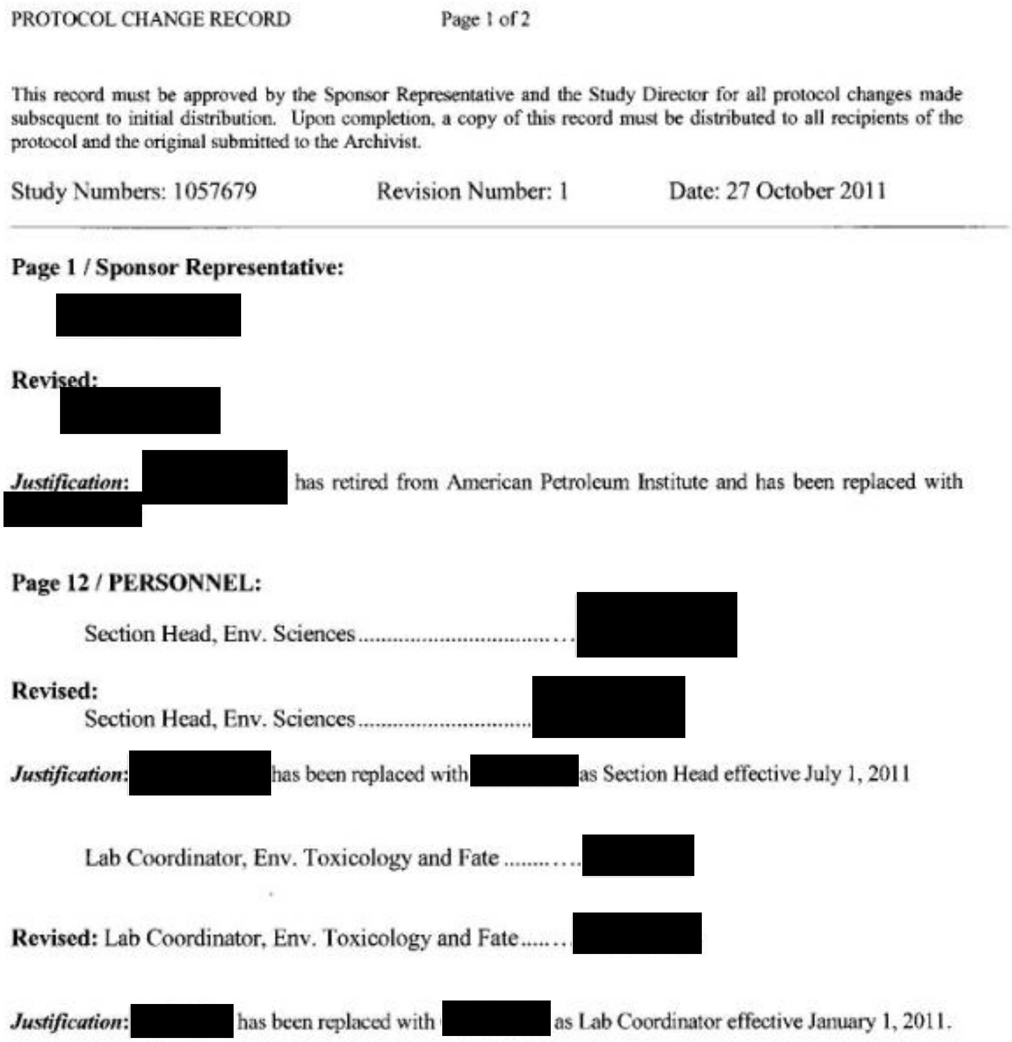
1. Organization for Economic Cooperation and Development (OECD), Guideline for Testing of Chemicals, Ready Biodegradability, 301F Manometric Respirometry Test (1992).
2. Organization for Economic Cooperation and Development, Principles of Good Laboratory Practice, C(97) 186/Final, 1997.
3. Organization for Economic Cooperation and Development (OECD), Guidelines for the Testing of Chemicals, Section 3, Introduction. (Adopted 23 March 2006)
4. United States Environmental Protection Agency (USEPA) Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards, 40 CFR Part 792, 1989.

PERSONNEL

Study Director ....
Sponsor Representative ....
API Study Monitor ....
Section Head, Env. Sciences ....
Lab Coordinator, Env. Toxicology and Fate...........
Lab Coordinator, Environmental Chemistry,
Principal Investigator Characterization (EMBSI)....
Study Technicians...
....
....
....
....
Quality Assurance Unit ....
Contract Administrator..

[redacted]

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



PROTOCOL CHANGE RECORD Page 1 of 2

This record must be approved by the Sponsor Representative and the Study Director for all protocol changes made subsequent to initial distribution. Upon completion, a copy of this record must be distributed to all recipients of the protocol and the original submitted to the Archivist.

Study Numbers: 1057679 Revision Number: 1 Date: 27 October 2011

Page 1 / Sponsor Representative:

[redacted]

Revised:

[redacted]

***Justification*:** [redacted] has retired from American Petroleum Institute and has been replaced with [redacted]

Page 12 / PERSONNEL:

Section Head, Env. Sciences.................................... [redacted]

Revised:
Section Head, Env. Sciences.............................. [redacted]

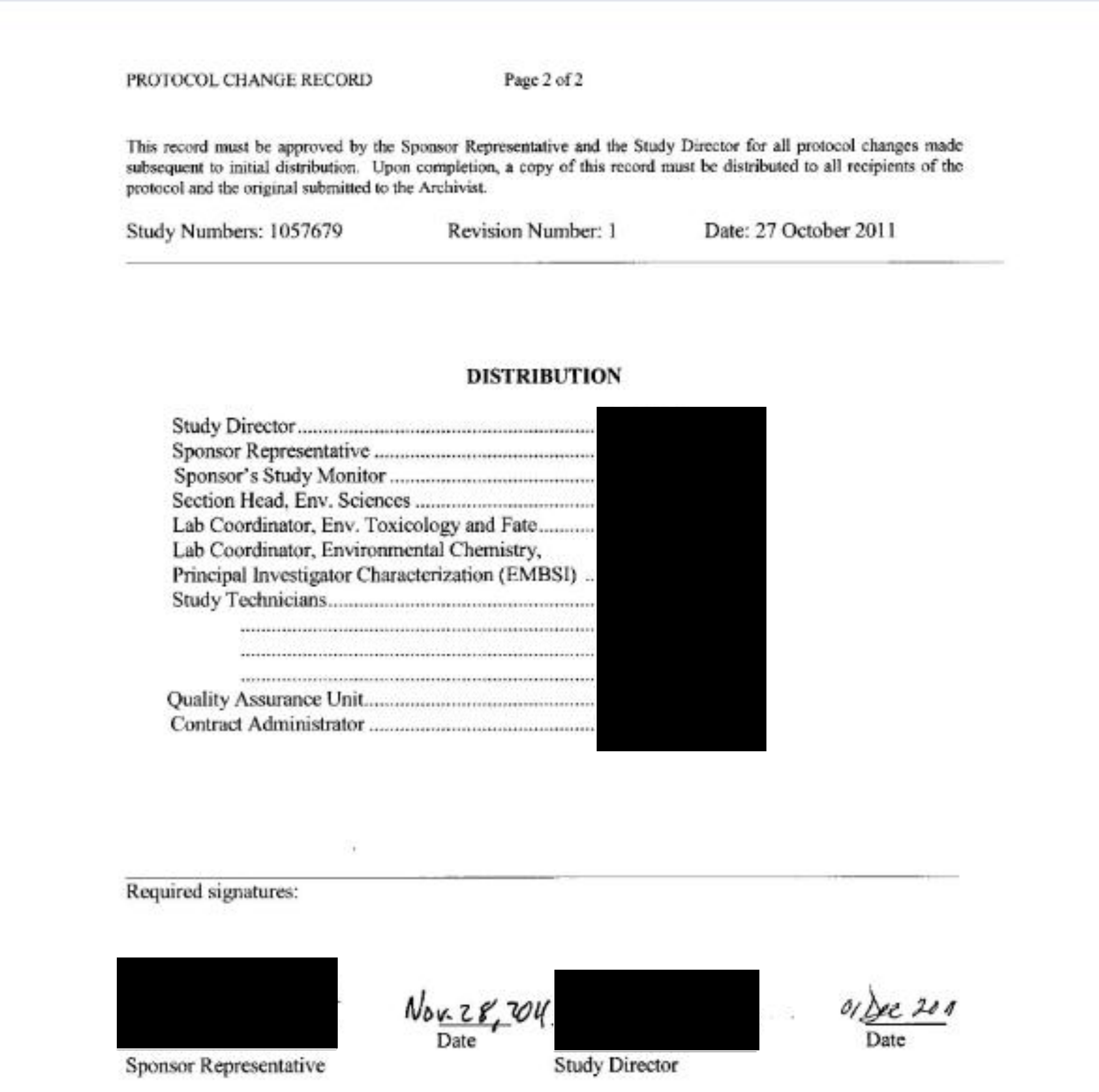
***Justification*:** [redacted] has been replaced with [redacted] as Section Head effective July 1, 2011

Lab Coordinator, Env. Toxicology and Fate ....…. [redacted]

Revised: Lab Coordinator, Env. Toxicology and Fate......… [redacted]

***Justification*:** [redacted] has been replaced with [redacted] as Lab Coordinator effective January 1, 2011.

APPENDIX F – PROTOCOL AND PROTOCOL REVISION (CONT'D)



PROTOCOL CHANGE RECORD Page 2 of 2

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Study Numbers: 1057679 Revision Number: 1 Date: 27 October 2011

DISTRIBUTION

Study Director ....
Sponsor Representative ....
Sponsor's Study Monitor ....
Section Head, Env. Sciences ....
Lab Coordinator, Env. Toxicology and Fate ....
Lab Coordinator, Environmental Chemistry,
Principal Investigator Characterization (EMBSI) ..
Study Technicians ....
....
....
....
Quality Assurance Unit ....
Contract Administrator ....

[redacted]

Required signatures:

[redacted] Nov. 28, 2011 [redacted] 01 Dec 2011

Sponsor Representative Date Study Director Date