STUDY TITLE

NOVAPLUS System (Fluido Novaplus SBM com Novawet II): Determination of Biodegradability in Seawater Using the Closed Bottle Test Method

DATA REQUIREMENT

OECD Guideline 306

AUTHOR

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STUDY INITIATION DATE

January 24, 2008

STUDY COMPLETION DATE

March 03, 2008

SPONSOR

M-I SWACO
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PERFORMING LABORATORY

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PROJECT IDENTIFICATION

ABC Study No. 63454

STUDY COMPLIANCE STATEMENT

This Compliance Statement is for ABC Study No. 63454 entitled, "NOVAPLUS System (Fluido Novaplus SBM com Novawet II): Determination of Biodegradability in Seawater Using the Closed Bottle Test Method," for M-I SWACO, Houston, Texas.

The Study Director for the above test herein confirms that, with the following exceptions, the study was performed in compliance with the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practices (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998.

The test and reference substances were not characterized under the aforementioned GLP practices.

These exceptions did not adversely affect the study integrity or the interpretation of the test results.

All original raw data, including the original protocol, were submitted to the Sponsor along with the final report. A copy of the final report, protocol, and all raw data from the study, as well as all original facility records, are kept on file in ABC Laboratories' archives.

Philip Sarff
Philip Sarff
Study Director
ABC Laboratories, Inc.

03 Mar. 2008
Date

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed ABC Study No. 63454 entitled, "NOVAPLUS System (Fluido Novaplus SBM com Novawet II): Determination of Biodegradability in Seawater Using the Closed Bottle Test Method," for M-I SWACO, Houston, Texas. The following audits/inspections were performed on this study.

Date of Study-Based Inspection	Phase Inspected	Date Reported to Study Director	Date Reported to Management
January 30, 2008	Procedure: Test Substance Weigh Out	January 31, 2008	February 01, 2008
February 27, 2008	Raw Data and Draft/Final Report	February 27, 2008	March 03, 2008

These audits indicate that the report is an accurate reflection of the study as performed by ABC Laboratories, Inc.

Jeri Hofen — 03Mar2008

Jeri Hofen
Quality Assurance Associate II
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Date

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STUDY SUMMARY

The primary objective of this study was to evaluate the biodegradability of the test substance, NOVAPLUS System (Fluido Novaplus SBM com Novawet II), in natural seawater at an initial test concentration of 5 mg/L, following OECD guideline 306. The test substance was exposed to the seawater inoculum under aerobic conditions for 18 days at approximately 20°C. The seawater was obtained from Gulf Specimen Marine Laboratory, Panacea, Florida. A reference substance treatment containing readily biodegradable sodium benzoate at a nominal concentration of 5 mg/L was concurrently tested to verify the viability of the microbial inoculum. A control treatment containing no test or reference substance was concurrently tested to measure the oxygen uptake caused by endogenous microbial respiration. Two BOD bottles from each treatment of 10 bottles were randomly removed after 0, 7, 14, and 18 days of incubation at 20°C and were measured for dissolved oxygen concentration and for pH on Days 0 and 18 (2 bottles for each treatment were not used).

Chemical oxygen demand (COD) was measured by the UV/VIS method. Five replicate samples of the test substance were prepared for the UV/VIS determination by weighing 1.5752, 1.0852, 1.0383, 1.2971, and 0.8264 mg of test substance, respectively, on cover slips and depositing the cover slips into Bioscience accu-TEST™ Standard Range COD vials with 2.5 mL of reagent water. The mean COD of the five replicates was 1.45 mg O_2/mg.

Biochemical oxygen demand (BOD) was calculated from the concentration of dissolved oxygen consumed in the test or reference substance bottles (mg O_2/L), corrected for that in the control, expressed as a fraction of the nominal testing concentration. BOD for the test substance reached a maximum of 1.21 mg O_2/mg of test substance on Day 7. BOD for the reference substance reached a maximum of 1.52 mg O_2/mg of reference substance on Day 7.

Percent degradation was calculated by expressing BOD as a percent of theoretical oxygen demand (ThOD) or chemical oxygen demand (COD). The COD for the test substance was determined to be 1.45 mg O_2/mg. The percent degradation for the test substance treatment on Days 7, 14, and 18 was 83.0, 79.6, and 69.8%, respectively. The test was terminated on Day 18 due to >60% biodegradation of the test substance on Day 7 and remaining above this level at Days 14 and 18. Since biodegradation of was greater than 60%, the test results indicate that NOVAPLUS System (Fluido Novaplus SBM com Novawet II) has the potential for biodegradation in a marine environment according to OECD Guideline 306. The ThOD for the reference substance was 1.67 mg O_2/mg. The percent degradation for the reference substance reached 91.1% by Day 7, verifying that the seawater inoculum was viable and active.

Bacterial plate count analyses indicated that the test substance was not toxic to the seawater inoculum at the testing concentration (5 mg/L).

1.0 INTRODUCTION

The purpose of this study was to evaluate the aerobic biodegradability of NOVAPLUS System (Fluido Novaplus SBM com Novawet II) in natural seawater containing mineral salts. The study was conducted as described in the ABC study protocol and alterations (two amendments and a deviation) entitled "NOVAPLUS System (Fluido Novaplus SBM com Novawet II): Determination of the Biodegradability in Seawater Using the Closed Bottle Test Method" (Appendix A), which was patterned after the Organization for Economic Cooperation and Development (OECD), Guideline for Testing of Chemicals, Method 306 (1).

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Test Substance

ABC Laboratories received a sample of the test substance, NOVAPLUS System, from M-I SWACO on January 18, 2008. The test substance was stored at room temperature. The following information pertains to the test substance.

Test Substance Name:	NOVAPLUS System
Portuguese Name:	Fluido Novaplus SBM com Novawet II
CAS Number:	Not Applicable (Mixture)
Lot Number:	Not Given
Expiration Date:	Not Given
Appearance:	Grey to Brown Liquid
Purity:	Tested as 100%
Composition:	

Product	Concentration
NOVAPLUS B	167.13 lbs/bbl
Calcium Chloride	33.6 lbs/bbl
HYDRATED LIME	6.0 lbs/bbl
NOVAMUL	6.0 lbs/bbl
NOVAWET II	2.0 lbs/bbl
VG SUPREME	1.5 lbs/bbl
M-I BR CLAYPLUS	5.0 lbs/bbl
HRP	0.5 lbs/bbl
WATER	87.36 lbs/bbl
ECOTROL RD	1.0 lbs/bbl
Calcium Carbonate 2-44	15.0 lbs/bbl
BARITE	73.9 lbs/bbl

Note: lbs/bbl = pounds per barrel

2.1.2 Reference Substance

A sample of the reference substance, sodium benzoate, was received from Fisher Scientific on April 05, 2005. The reference substance was stored at room temperature. The following information pertains to the reference substance.

CAS Number:	532-32-1
Molecular Formula:	$C_7H_5O_2Na$
Molecular Weight:	144.11 g/mol
Lot Number:	041447
Chemical Purity:	100.4%
Expiration Date:	None Given
Appearance:	White Powder
Water Solubility:	soluble

2.1.3 Reagent Water

Reagent water was purified, deionized, and filtered using a Millipore Milli-Q Water Purification System or a Labconco Water Pro PS unit.

2.1.4 Test Inoculum

The microbial inoculum used in this test was seawater collected by Gulf Specimen Marine Laboratory in Panacea, Florida, on January 23, 2008. Gulf Specimen provided approximately 20 L of seawater from Dickerson Bay and recorded the temperature of the seawater before shipment as 12°C. A data logger was included in the package with the seawater to continuously monitor the temperature during shipment. The seawater was transferred to a carboy and was stirred and aerated in an environmental chamber at 20°C until preparation of the test medium.

2.1.5 Test Medium

On January 29, 2008, concentrated mineral salts stock solutions were added to the seawater to provide essential mineral nutrients and trace elements necessary to sustain the seawater inoculum throughout the test period. The test medium was stirred and aerated in an environmental chamber at 20°C for one day until initiation of the test. Prior to test initiation, the test medium dissolved oxygen concentration and pH were measured to be 8.72 mg O_2/L and 7.82, respectively.

2.1.6 Test Vessels

Clean, 300-mL BOD bottles with glass stoppers were used for this test.

2.2 Methods

2.2.1 Preparation of Reference Substance Stock Solutions

A 1.00-mg/mL stock solution of the reference substance was prepared by weighing 500.7 mg of sodium benzoate to a 500-mL class A volumetric flask and diluting to volume with reagent

water. This stock solution was refrigerated when not in use. This solution was used in the determination of chemical oxygen demand of the test substance.

A 1.00-mg/mL stock solution of the reference substance was prepared by weighing 500.9 mg of sodium benzoate to a 500-mL class A volumetric flask and diluting to volume with reagent water. This solution was used on the day of preparation in the biodegradation testing.

2.2.2 Determination of Chemical Oxygen Demand

Since the test substance composition was unknown, the theoretical oxygen demand could not be calculated. Thus, the chemical oxygen demand (COD) was measured by the closed reflux, colorimetric method described in *Standard Methods for the Examination of Water and Wastewater* (2).

The test substance was mixed for 15 minutes using an electric drill and small blade mixing propeller before being dosed directly to the COD vials. Five replicate samples of the test substance were prepared for the closed reflux reaction by weighing 1.5752, 1.0852, 1.0383, 1.2971, 0.8264 mg of test substance onto cover slips and depositing the cover slips into Bioscience accu-TEST™ Standard Range COD vials with 2.5 mL of reagent water.

Two blank samples were prepared by adding 2.5 mL of reagent water to Bioscience accu-TEST™ Standard Range COD vials. One of the blank samples was digested on the COD reactor, and the second was kept at ambient temperature as an undigested blank.

A 1.06-mg/mL stock solution of potassium hydrogen phthalate (KHP) was prepared by weighing 105.8 mg of KHP into a 100-mL Class A volumetric flask and diluting to volume with reagent water. Since the COD of KHP is 1.176 mg O_2/mg, the COD of the 1.06-mg/mL stock solution was 1.25 mg O_2/mL. Samples of the standard were prepared for the closed reflux reaction by adding 0.10, 0.30, 0.70, 1.0, 1.4, and 1.8 mL of the 1.06-mg/mL KHP stock solution and 2.4, 2.2, 1.8, 1.5, 1.1, and 0.70 mL of reagent water, respectively, to Bioscience accu-TEST™ Standard Range COD vials. The calculated COD of the standards were 0.125, 0.375, 0.875, 1.25, 1.75, and 2.25 mg O_2.

Quality control samples were prepared to verify the analysis. Triplicate reference samples were prepared for the closed reflux reaction by adding 1.0 mL of the 1.00-mg/mL sodium benzoate stock solution and 1.5 mL of reagent water to Bioscience accu-TEST™ Standard Range COD vials. The theoretical oxygen demand (ThOD) of the quality control samples was 1.67 mg O_2/mg.

The prepared COD samples were shaken and placed on a Bioscience COD reactor for two hours at approximately 150°C. The samples were allowed to cool to ambient temperature, and then the absorbances were measured at 600 nm on a Genesys 20 Spectrophotometer. The absorbance was initially zeroed using the undigested blank. The absorbance of all other samples was measured in comparison to the undigested blank. Sample COD (mg O_2/mg) values were calculated by interpolation of sample absorbance with linear regression of the standards' absorbance versus COD (mg O_2).

2.2.3 Preparation of the Bulk Testing Solutions

Bulk testing solutions for the control and test treatment were prepared by adding 3,980 mL of test medium to each of two 5-L Nalgene carboys containing 20 mL of reagent water, bringing the total volume to 4,000 mL. The reference substance treatment was dosed at a nominal concentration of 5 mg/L by adding 20 mL of the 1.00-mg/mL reference substance stock solution and 3,980 mL to a 5-L Nalgene carboy to bring the total volume to 4,000 mL. The prepared bulk solutions were stirred using Teflon-coated magnetic stir bars and insulated magnetic stir plates.

2.2.4 Preparation and Incubation of the BOD Bottles

Immediately prior to use, the test substance was mixed for 15 minutes using an electric drill and small blade mixing propeller. Approximately 1.5-mg aliquots of the test substance were weighed onto coverslips, which were then placed into the test BOD bottles. Coverslips with no test substance were added to the control and reference BOD bottles.

Each bulk testing solution was transferred into ten BOD bottles by draining from the corresponding 5-L Nalgene carboy through the spigot. All BOD bottles were sealed with glass stoppers, leaving no headspace of air and labeled with study number, treatment, replicate number, date of initiation, and initials (Study Director's and preparer's).

The ten BOD bottles within each treatment were randomly designated for sampling in duplicate utilizing a random number table. Two bottles randomly designated for Day 0 analysis were removed from each of the treatment groups. The remaining bottles were placed on an orbital shaker in an environmental chamber set at 20°C and maintained in darkness under a black cloth. Temperature in the environmental chamber was continuously measured.

2.2.5 Dissolved Oxygen and pH Measurements

Dissolved oxygen concentrations were measured using a WTW Oxi 330i or a YSI Model 58 dissolved oxygen meter. This dissolved oxygen probe is sensitive enough to measure dissolved oxygen values below 0.5 mg O_2/L. Test solution pH was measured using an Accumet model AR50 pH meter. Dissolved oxygen was measured for the two replicates from each of the three treatments on Days 0, 7, 14, and 18. pH measurements were taken at Day 0 and Day 18.

2.2.6 Microbial Evaluation

Bacterial plate counts were performed on the test medium and seawater at initiation and on one of the duplicate BOD bottles from each treatment at Day 18 and were patterned after methods described in *Standard Methods for the Examination of Water and Wastewater* (3). The plates were incubated at 26 $\pm$ 2°C for four to seven days before counting the number of colonies on plates with greater than 30, but fewer than 300 colonies. The number of colonies at the dilution coming closest to 300 colonies was used to calculate colony forming units (CFU)/mL.

2.3 Calculations

2.3.1 Calculation of Theoretical Oxygen Demand (ThOD)

The theoretical oxygen demand was calculated for the reference substance based on their elemental composition using the following equations.

For substances with no nitrogen or for substances with nitrogen and where nitrogen is not utilized through nitrification,

$$\mathrm{ThOD}_{NH3} = \frac{16[2C + \frac{1}{2}(H - Cl - 3N) + 3S + \frac{5}{2}P + \frac{1}{2}Na - O]}{\text{Molecular Weight (g/mol)}}$$

The variables C, H, Cl, N, S, P, Na, and O are each element's number of atoms in the molecule. The calculated ThOD was indicated in mg of oxygen per mg of substance.

The reference substance, sodium benzoate, contains no nitrogen, and the ThOD_{NH3} was 1.67 mg O_2/mg.

2.3.2 Calculation of Chemical Oxygen Demand (COD)

The COD of the sample (mg O_2) was determined from the standard curve prepared from the KHP standards as shown in the equation below:

$$Y = mX + b$$

Where:

Y = Absorbance
m = the slope of the line from the calibration curve
X = sample COD (mg O_2)
b = the Y-intercept of the calibration curve

The sample COD (mg O_2) was then divided by the sample amount to determine the amount of oxygen per amount of test substance. Calculations were performed using Microsoft Office Excel 2003.

2.3.3 Calculation of Biochemical Oxygen Demand (BOD)

The BOD was calculated by the following equation:

$$BOD = \frac{DO_T - DO_B}{C_T}$$

Where:

DO_T = mean DO uptake in the test or reference substance bottles (mg O_2/L)
DO_B = mean DO uptake in the inoculum blank bottles (mg O_2/L)
C_T = testing concentration for the test or reference substances (mg/L)

The calculated BOD was indicated in mg of oxygen per mg of test substance.

2.3.4 Calculation of Percent Degradation

The percent degradation based on ThOD or COD was calculated by the following equation:

$$\text{Percent Degradation} = \frac{BOD}{\text{ThOD or COD}} \times 100\%$$

Where:

COD = 1.45 mg O_2/mg for the test substance treatment, or
ThOD = 1.67 mg O_2/mg for the reference substance treatment

3.0 RESULTS AND DISCUSSION

3.1 Test Water

The seawater sample was collected at the surface of a coastal area known as Dickerson Bay. The minimum and maximum recorded temperature of the seawater during shipment was 10.6 and 20.5°C, respectively. Upon arrival at ABC Laboratories on January 24, 2008, the salinity of the seawater was measured as 29.0 parts per thousand (ppt). The seawater was pretreated by sedimentation and decanting.

3.2 Ingredients of the Test Medium

Mineral salts stock solutions were added to the seawater to provide essential mineral nutrients and trace elements necessary to sustain the seawater inoculum throughout the test period. The test medium was prepared using 1 mL of each of the solutions shown in Table 1 per liter of seawater. The test medium was stirred and aerated in an environmental chamber at approximately 20°C until initiation of the test.

3.3 Chemical Oxygen Demand of the Test Substance

The mean COD of the test substance was determined to be 1.45 mg O_2/mg (Table 2). The correlation of the standard curve was 0.9997. The mean COD of the reference sample was calculated to be 1.67 mg O_2/mg, which is equivalent to the ThOD.

3.4 Endogenous Oxygen Respiration in the Control Treatment

Dissolved oxygen (DO) concentrations in the control BOD bottles decreased from a mean of 8.09 mg O_2/L on Day 0 to a mean of 5.87 mg O_2/L on Day 18 (Table 3). The purpose of the controls was to provide a measurement of the dissolved oxygen consumed due to endogenous respiration of the microbial inoculum. As a percent of the initial measured dissolved oxygen concentration, 8.09 mg O_2/L, the total oxygen consumed was a maximum of 27% (difference of dissolved oxygen values on Days 0 and 18 divided by dissolved oxygen value at Day 0 for the control treatment). This value was within the recommendation stated in the guideline (1) that the oxygen consumed by the control should not exceed 30% of the initial concentration.

3.5 Biodegradation of the Reference Substance

The reference substance achieved 91.1% biodegradation by Day 7 of the test (Table 4, Figure 1 and Figure 2). Based on this result, the reference substance met the acceptability criteria defined in the protocol (>60% biodegradation within 10 days) and confirmed that the microbial inoculum was viable and active.

3.6 Biodegradation of the Test Substance

The test substance, NOVAPLUS System, reached a maximum biodegradation of 83.0% on Day 7 of the study. Since biodegradation of NOVAPLUS System was greater than 60%, the test results are positive for biodegradation in a marine environment according to OECD Guideline 306 (1). It was noted that the DO at Day 18 for one replicate was higher than other replicates at Day 14 and 18 (1.30 mg O_2/L versus <0.25 mg O_2/L). Since this value would also correspond to biodegradation of >60%, no further investigation was performed. These results are shown in Table 4 and graphically in Figure 1 and Figure 2.

3.7 Microbial Evaluation

Bacterial plate-count results of 1.70×10^5 and 1.10×10^5 CFU/mL for the aerated seawater and the Day 0 test medium confirmed the microbial inoculum was viable and active at initiation of the study. Bacterial plate counts on Day 18 (Table 5) indicate the microbial inoculum remained viable and active through the end of the test in each treatment. Bacterial plate count results of the test substance treatment show that the test substance was not toxic to the microbial inoculum at the testing concentration (5 mg/L).

3.8 Temperature

The mean and standard deviation of the temperature measurements were 19.8 $\pm$ 0.2°C from Day 0 to the end of the 18-day test. The temperature of the environmental chamber ranged from 19.3 to 20.6°C during this time.

3.9 pH Measurements

All pH values were in an acceptable range for biological systems (Table 6).

4.0 CONCLUSIONS

The percent degradation for the test substance treatment on Days 7, 14, and 18 was 83.0, 79.6, and 69.8%, respectively. The test was terminated on Day 18 due to >60% biodegradation of the test substance on Days 7 and 14. Since biodegradation of was greater than 60%, the test results indicate that NOVAPLUS System (Fluido Novaplus SBM com Novawet II) has the potential to biodegrade in a marine environment according to OECD Guideline 306 (1). The percent degradation of the reference substance, sodium benzoate, reached 91.1% on Day 7, meeting the test acceptability criteria. The results from bacterial plate count analyses indicated that the test substance was not toxic to the seawater inoculum at the testing concentration of 5 mg/L.

There were no difficulties encountered that would affect the integrity of the study.

5.0 REFERENCES

(1) Organization for Economic Cooperation and Development (OECD). July 17, 1992. OECD Guideline for the Testing of Chemicals. Biodegradability in Seawater, OECD Guideline No. 306.

(2) Standard Methods for the Examination of Water and Wastewater. 20th Edition. 1998. American Public Health Association. Part 5220 Chemical Oxygen Demand (COD).

(3) American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Part 9215 B. Pour Plate Method.

Table 1. Ingredients of Mineral Salts Medium

Solution	Compound	Stock Solution Concentration (g/L) In Reagent Water
A[1]	KH_2PO_4	8.47
	K_2HPO_4	21.7
	$Na_2HPO_4 \bullet 7H_2O$	50.3
	NH_4Cl	0.507
B	$CaCl_2 \bullet 2H_2O$	36.62
C	$MgSO_4 \bullet 7H_2O$	22.5
D	$FeCl_3 \bullet 6H_2O$	0.253
	Concentrated HCl	1 drop

[1] pH of solution A was 7.41.

The test medium was prepared using 1 mL of each of the above solutions per liter of seawater.

Table 2. Determination of Chemical Oxygen Demand

Sample ID	Rep.	Measured Absorbance (λ = 600 nm)	COD (mg O_2)	Sample Amount Added[1]	Conc. Of Sample Added (mg/mL)	Sample COD (mg O_2/mg)
Sodium Benzoate (Quality Control)	1	0.264	1.669	1.00	1.00	
	2	0.264	1.669	1.00	1.00	
	3	0.264	1.669	1.00	1.00	
	Mean		1.669	1.00	1.00	1.67[2]
NOVAPLUS System	1	0.356	2.254	1.5752	---	1.43
	2	0.254	1.605	1.0852	---	1.48
	3	0.238	1.504	1.0383	---	1.45
	4	0.292	1.847	1.2971	---	1.42
	5	0.194	1.224	0.8264	---	1.48
					Mean:	1.45
					STDEV:	0.03
					CV:	2 %

Values calculated using un-rounded numbers.

COD (mg O_2) = (Measured Absorbance – Y-intercept) / slope
y-intercept = 1.50×10^{-3}
Slope = 0.1573
r^2 = 0.9997

Sample COD (mg O_2/mg) = COD / Sample Amount (mg)

[1] Reference (Sodium Benzoate) added by volume (mL). Test substance (NOVAPLUS System) added by weight (mg).

[2] COD of quality control represents 100% of the ThOD, 1.67 mg O_2/mg.

Table 3. Endogenous Oxygen Respiration in the Control Treatment

Sampling Day	Dissolved Oxygen (mg O_2/L)			Mean Oxygen Uptake (mg O_2/L)
	Replicate 1	Replicate 2	Mean	
0	8.06	8.12	8.09	---
7	7.01	7.00	7.01	1.08
14	6.33	5.73	6.03	2.06
18	5.83	5.91	5.87	2.22

Note: Intermediate values are not rounded.

Table 4. Biodegradation of Reference and Test Substances

Day	Rep	Dissolved Oxygen (mg O_2/L)		Mean Oxygen Uptake (mg O_2/L)		BOD (mg O_2/mg)		Percent Degradation	
		Reference	Test	Reference	Test	Reference	Test	Reference	Test
0	1	8.82	8.19	---	---	---	---	---	---
	2	8.83	8.12						
	Mean	8.83	8.16						
7	1	0.13	0.90	8.69	7.18	1.52	1.21	91.1	83.0
	2	0.15	1.06						
	Mean	0.14	0.98						
14	1	0.12	0.11	8.72	7.98	1.33	1.16	79.8	79.6
	2	0.10	0.24						
	Mean	0.11	0.18						
18	1	0.11	1.30	8.72	7.44	1.30	1.01	77.8	69.8
	2	0.10	0.13						
	Mean	0.11	0.72						

Note: Intermediate values are not rounded.

Table 5. Bacterial Plate Count Results

Test Medium at Initiation (CFU/mL)	Control at Day 18 (CFU/mL)	Reference at Day 18 (CFU/mL)	Test at Day 18 (CFU/mL)
1.10×10^5	3.4×10^4	8.5×10^4	3.3×10^4

Note: Plates at initiation incubated for seven days, while the plates at Day 18 were incubated for four days.

Table 6. pH Measurements

Day	Replicate	Treatment Group		
		Control	Reference Substance	Test Substance
0	1	7.72	7.92	7.96
	2	7.87	7.90	7.98
18	1	7.62	7.27	7.43
	2	7.64	7.26	7.38

Figure 1. Oxygen Uptake vs. Time for the Control, Test Substance, and Reference Substance Treatments

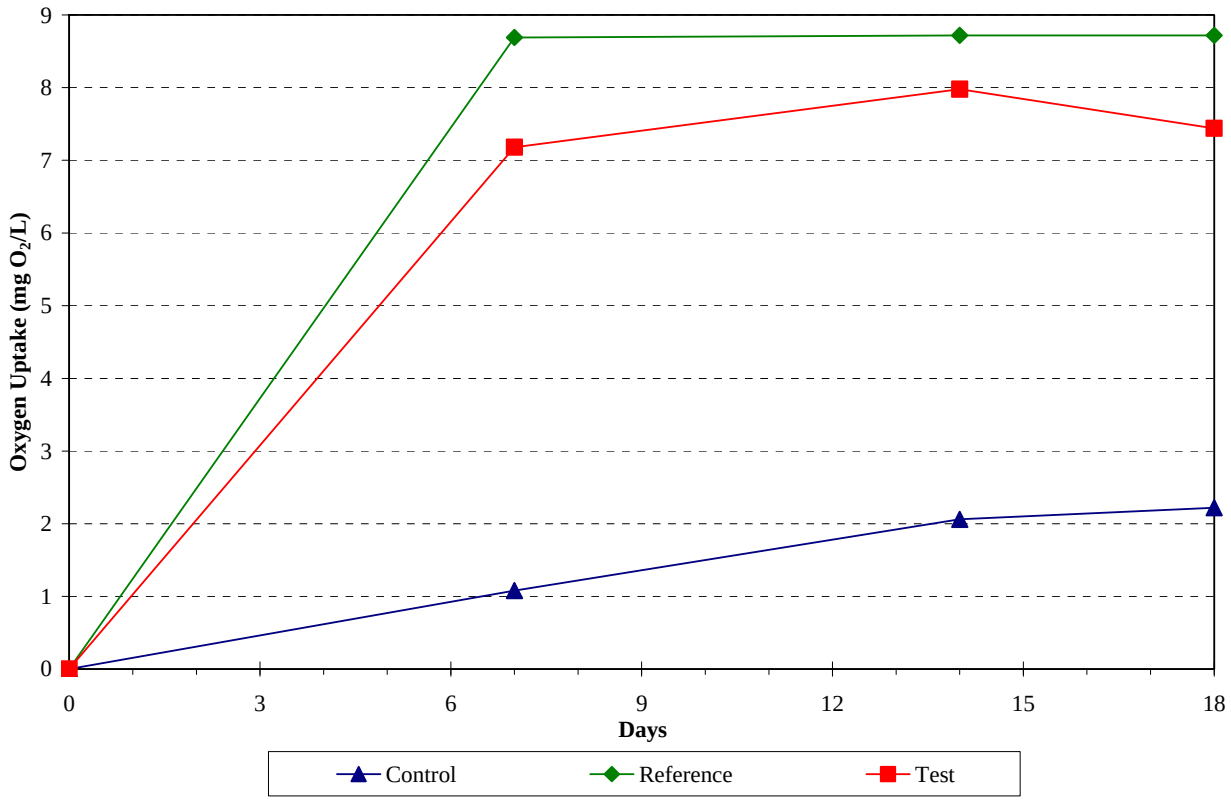
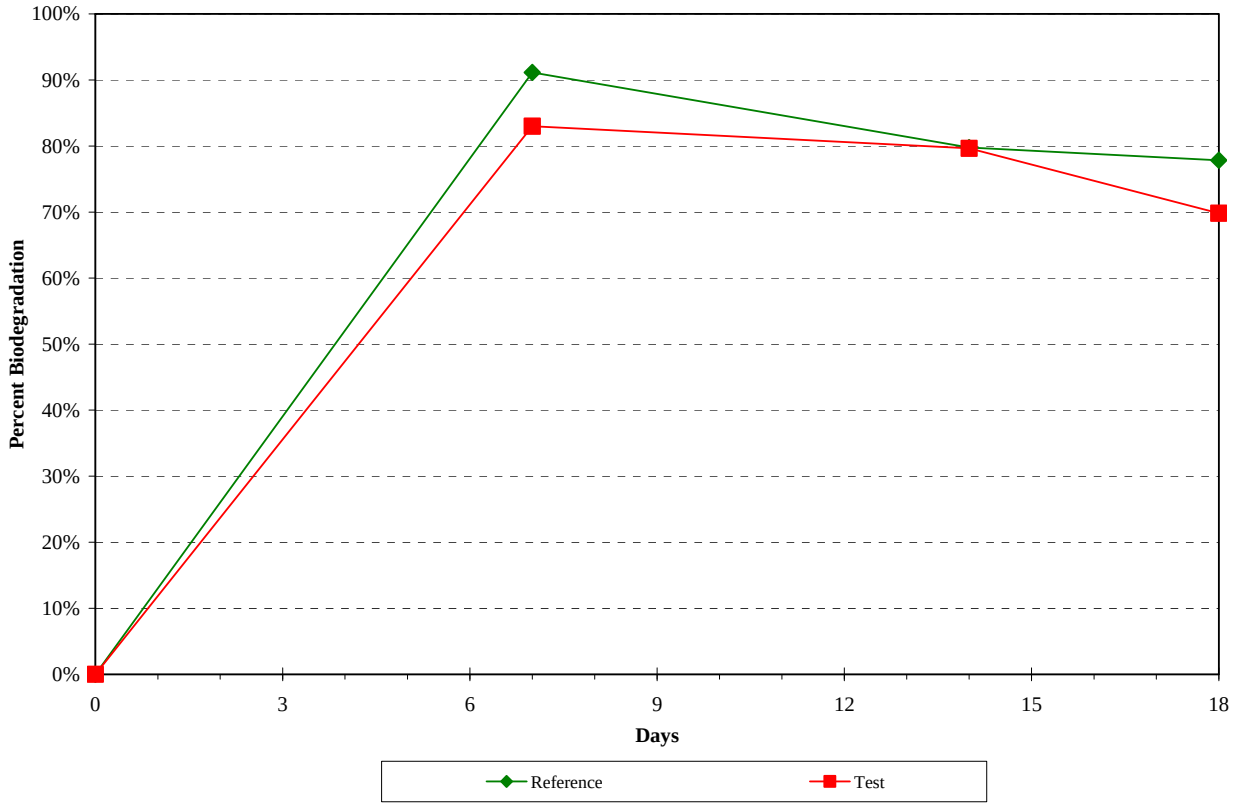


Figure 2. Percent Degradation vs. Time for the Test and Reference Substances



APPENDIX A – PROTOCOL AND ALTERATIONS

NOVAPLUS System: Determination of Biodegradability in Seawater Using the Closed Bottle Test Method

ABC Study No. 63454

This protocol complies with OECD Guideline 306

This protocol is based on ABC Generic Protocol No. E818.

1.0 STUDY TITLE

NOVAPLUS System: Determination of Biodegradability in Seawater Using the Closed Bottle Test Method

2.0 OBJECTIVE

The primary objective of this study is to evaluate the aerobic biodegradability of a test substance in natural seawater containing mineral salts. The microbial flora in the seawater sample will be the only source of microbes. The specific objectives of the study are: 1) to evaluate the biodegradability potential of the test material in an aerobic, aqueous medium via dissolved oxygen utilization; and 2) to determine the oxygen utilization of a reference chemical in order to assess the viability of the test inoculum.

3.0 STUDY SPONSOR

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TEL: 281-561-1374 FAX: 281-561-1342
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4.0 TESTING FACILITY AND STUDY DIRECTOR ADDRESS

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5.0 PROPOSED SCHEDULE

PROPOSED EXPERIMENTAL START DATE: January 2008
PROPOSED EXPERIMENTAL COMPLETION DATE: March 2008

6.0 TEST PROTOCOL

This test protocol is based on OECD Guideline for the Testing of Chemicals, Method 306 (1).

7.0 QUALIFYING STATEMENTS

The closed bottle test is considered amenable to testing volatile test substances and substances of low water solubility, as well as non-volatile and water soluble substances.

This method is not suitable for testing substances that are inhibitory to bacteria or are chemically unstable under the conditions of the test. Provisions may be made to investigate inhibitory effects, but will be performed as an additional scope.

The OECD 306 method (1) was established for pure substances or those containing homologues. Whenever possible, test substances that are mixtures should not be tested, but the individual components should be tested in its place.

8.0 TEST AND REFERENCE SUBSTANCES

8.1 Test Substance

The test substance will be NOVAPLUS System. The following sample information and chemical/physical properties should be provided with the test substance sample or before its shipment: batch/lot number, sample expiration date, physical description, purity (including certificate of analysis), stability, suggested storage conditions, water and organic solvent solubility, vapor pressure, available toxicity information, a Material Safety Data Sheet (MSDS) or its equivalent, and handling precautions. In addition, the empirical formula of the test substance is required to determine the theoretical oxygen demand (ThOD). If the ThOD cannot be provided or calculated, the Chemical Oxygen Demand (COD) of the test substance should be provided or generated as additional scope to serve as the reference point.

Prior to each use, the test substance will be appropriately mixed to ensure homogeneity.

8.2 Reference Substance

A reference substance, such as sodium benzoate, is used to verify the inoculum's activity in the test systems. Information on the reference substance will be obtained from the supplier.

8.3 Sample Characterization and Retention

Characterization, stability, and solubility studies will be the responsibility of the Sponsor unless otherwise contracted to ABC Laboratories, Inc. The test substance will be properly disposed of following completion of its use at ABC Laboratories, Inc., unless other arrangements are made with Sponsor. Archival of a retention sample, if required, will be the Sponsor's responsibility.

8.4 Test Substance Preparation/Addition

For water-soluble test substances, a stock solution will be prepared at a known concentration, which will be documented in the raw data. Stock solutions will be based on the percentage of active ingredient in test substance, if known. If the percentage of active ingredient is unknown, aqueous stock solutions will be prepared at a known concentration on a total product weight basis.

The pH of the stock solution will be measured. pH adjustments may be necessary depending on the dose volume and/or when the dose solution is extremely basic (pH >10) or acidic (pH <3) so as to not adversely affect the microbial population, but will only be made after consultation with the Sponsor.

Insoluble test chemicals can be dosed directly on a gravimetric basis by adding the test substance to a glass weigh boat or glass coverslip. Alternatively, and as an additional scope, insoluble test substances can be dosed using silica gel as an inert carrier (Attachment 1). Biodegradation for some water insoluble test substances has been shown to be more rapid and more complete when the test substance was adsorbed to an inert carrier (2).

9.0 MATERIALS

9.1 Test Water

Natural seawater will be collected and shipped to ABC Laboratories for testing. The temperature of the seawater sample will be measured at time of collection. The seawater sample will be transported within one to two days of collection in a manner such that the temperature of the seawater during transport approximates the temperature at collection, and should be similar to the testing temperature.

If the dissolved organic carbon (DOC) of the seawater sample is found to be high, it is believed that the blank BOD after 28 days will be more than 30% of that of the reference substances, or the temperature at collection is greater than ±5°C from the test temperature, the seawater will be aged for up to seven days prior to use. The seawater will be aged by storing it under aerobic conditions at the test temperature and in the dark or in diffuse light.

Prior to use, the seawater may be pretreated to remove coarse particles (if present).

9.2 Test Vessels

Clean (i.e., free from organic and toxic matter) 250-300 mL BOD bottles with glass stoppers will be used as test vessels. Glassware will be cleaned by adding 5-10 mL of a wash solution (2.5 g iodine + 12.5 g KI per liter of 1% w/v sulfuric acid) to the bottle and shaking well. The glassware will be allowed to stand for 15 minutes and then will be rinsed thoroughly with reagent water. The glassware will then be ashed

in an oven.

9.3 Oxygen Electrode

A YSI Model 54 Dissolved Oxygen Meter or equivalent with a BOD oxygen probe will be used to measure dissolved oxygen concentrations within the test vessels.

9.4 Environmental Chamber

The study will be conducted in a walk-in chamber at 20 ± 1°C in the dark. Laboratory lighting will be permitted within the chamber for short periods of time to allow for system monitoring, water quality measurements, and sample collection. The temperature of the environmental chamber will be recorded continuously for the duration of the test.

10.0 TEST PROCEDURES

10.1 Test Medium

10.1.1 Stock Solutions

Phosphate buffer solution: Approximately (i.e., within ±0.05 g) 8.50 g KH_2PO_4, 21.75 g K_2HPO_4, 33.30 g $Na_2HPO_4 \cdot 2H_2O$, and 0.50 g NH_4Cl will be dissolved in and diluted to 1 L with reagent water. The pH will be adjusted to 7.4 ± 0.2 with 1.0 N HCl or NaOH.

Calcium chloride solution: Approximately (i.e., within ±0.05 g) 27.50 g anhydrous $CaCl_2$ or 36.40 g $CaCl_2 \cdot 2H_2O$ will be dissolved in and diluted to 1 L with reagent water.

Magnesium sulfate solution: Approximately (i.e., within ±0.05 g) 22.50 g $MgSO_4 \cdot 7H_2O$ will be dissolved in and diluted to 1 L with reagent water.

Ferric chloride solution: Approximately (i.e., within ±0.02 g) 0.25 g $FeCl_3 \cdot 6H_2O$ will be dissolved in and diluted to 1 L with reagent water. One drop of concentrated HCl or 0.4 g EDTA will be added so that the solution may be stored.

Molar equivalents of chemicals may be used. The above volumes may be adjusted to make more or less, as necessary.

10.1.2 Test Medium Preparation

The test medium will be prepared by diluting 1 mL of phosphate buffer, calcium chloride, magnesium sulfate, and ferric chloride solutions per liter of pre-treated seawater. An appropriate volume of test medium will be prepared.

The test medium will be vigorously stirred and aerated (>250 mL/minute) for at least one hour. If the dissolved oxygen (DO) is <7 mg/L, stirring and aeration should be continued until the DO is >7 mg/L. The test medium will then be moderately stirred and aerated at 20°C until use. The DO and pH of the test medium will be measured immediately prior to use. All transfers and filling operations of the air-saturated medium will be conducted bubble-free (e.g., by siphoning).

10.2 Preparation of Test Bottles

To each of three 5-L carboys, an appropriate volume of the prepared test medium will be added. Test and reference substance will be added to the carboys as indicated below:

1 Carboy - test medium blank; no test or reference substance
1 Carboy - reference substance at 2 to 5 mg/L
1 Carboy - If soluble: test substance at 2 to 10 mg/L. If insoluble and test substance is gravimetrically added to the BOD bottles, composition in carboy will be the same as that of the test medium blank.

The concentration of test substance will be based on solubility, available toxicity information, and ThOD or COD. The reference substance will be added at the same concentration as the test substance, but not more than 5 mg/L. All carboys will be mixed thoroughly to ensure uniformity of the mixture.

At the Sponsor's request, an additional carboy may be set up to check for possible inhibitory effects of the test substance on the inoculum if toxicity is not known. This toxicity carboy will contain the inoculated test medium and both the test (if soluble) and reference substances at the respective concentrations. If the test substance is not soluble, the test substance will be added gravimetrically.

At the Sponsor's request, an additional carboy may be set up to check for abiotic mechanisms, such as hydrolysis or adsorption. This abiotic control carboy will contain the inoculated test medium, the test (if soluble) substance, and mercury chloride (50-100 mg/L) to stop the microbial activity. If the test substance is not soluble, the test substance will be added gravimetrically.

Each bottle will be filled leaving no headspace by the addition of the solutions from the corresponding carboys. Any air bubbles in the BOD bottles will be tapped out, and each bottle stoppered. Each sample bottle will be uniquely labeled.

10.3 Dissolved Oxygen and pH Measurements

Duplicate bottles from each treatment will be randomly designated for each sampling day. Immediately after the BOD bottles are filled, two samples from each treatment

will be taken for DO and pH measurements at day 0. The remaining bottles will be placed in an environmental chamber at 20 ± 1°C. Duplicate bottles from each testing group will be removed at test termination (day 28) and on two sampling points between initiation and termination (e.g., days 5 and 15) for DO and pH measurements. Periodically during incubation, the bottles will be agitated and the bottle funnels will be filled with reagent water.

10.4 Microbial Plate Counts

An aliquot of the test medium will be evaluated at day 0 for microbial activity by conducting a standard plate count (3). Aliquots from one BOD bottle of each treatment (e.g. control, reference, and test) will also be evaluated for microbial activity at termination to demonstrate that the test substance was not toxic to the microbial inoculum.

10.5 Nitrate/Nitrite Analysis

If the test substance contains nitrogen, the final concentration of nitrate and nitrite may be measured in all samples at the end of the test, so that the calculated degree of biodegradation can be corrected if nitrification has taken place.

11.0 CALCULATIONS AND STATISTICS

11.1 Calculation of Theoretical Oxygen Demand (ThOD)

The theoretical oxygen demand will be calculated for the test substance based on its elemental composition using the following equations.

For substances with no nitrogen or for substances with nitrogen and where nitrogen is not utilized through nitrification,

$$\mathrm{ThOD}_{NH3} = \frac{16[2C + \frac{1}{2}(H - Cl - 3N) + 3S + \frac{5}{2}P + \frac{1}{2}Na - O]}{\text{Molecular Weight (g/mol)}}$$

For substances with nitrogen and where nitrogen is completely utilized through nitrification,

$$\mathrm{ThOD}_{NO3} = \frac{16[2C + \frac{1}{2}(H - Cl) + \frac{5}{2}N + 3S + \frac{5}{2}P + 1/2Na - O]}{\text{Molecular Weight (g/mol)}}$$

The variables C, H, Cl, N, S, P, Na, and O are each element's number of atoms in the molecule. The calculated ThOD is indicated in mg of oxygen per mg of test substance.

If the test substance contains nitrogen, both equations will be used. The reference substance, sodium benzoate, contains no nitrogen, and the ThOD_{NH3} is

1.67 mg O_2/mg.

11.2 Calculation of Biochemical Oxygen Demand (BOD)

The BOD is calculated by the following equation:

$$BOD = \frac{DO_T - DO_B}{C_T}$$

Where:

DO_T	=	mean DO uptake in the test or reference substance bottles (mg O_2/L)
DO_B	=	mean DO uptake in the inoculum blank bottles (mg O_2/L)
C_T	=	testing concentration for the test and reference substances (mg/L)

The calculated BOD is indicated in mg of oxygen per mg of test substance.

11.3 Calculation of Percent Degradation

The percent degradation based on either ThOD or COD (when ThOD can not be determined) is calculated by the following equation:

$$\text{Percent Degradation} = \frac{BOD}{\text{ThOD or COD}} \times 100$$

If the test substance contains nitrogen, percent degradation will be calculated using both $ThOD_{NH3}$ and $ThOD_{NO3}$. If the test substance's classification of biodegradability is not the same when percent biodegradation is calculated using the different ThOD values (e.g., readily biodegradable using $ThOD_{NH3}$ and inherently biodegradable using $ThOD_{NO3}$), the final concentration of nitrate and nitrite will be measured in all samples. The oxygen consumed through nitrification will be determined from the change in concentration of nitrate and nitrite. The oxygen consumed in the formation of nitrate is 4.57 multiplied by the increase in concentration of nitrate-N, and the oxygen consumed in the formation of nitrite is 3.43 multiplied by the increase in concentration of nitrite-N. The total oxygen consumed will be summed and then subtracted from the DO_T prior to calculating BOD. The corrected value for oxygen consumption due to C-oxidation will then be compared with $ThOD_{NH3}$.

No bias in data is anticipated in the conduct of this study. No specific statistical tests will be used for this study.

12.0 TEST ACCEPTABILITY CRITERIA

For the test to be valid the following criteria should be met: (A) oxygen depletion in the test medium blank bottles should not exceed 30% of the initial oxygen concentration; (B) the residual concentration of oxygen in the BOD bottles should not fall below 0.5 mg/L at any time unless the method used for measuring the dissolved oxygen uptake is accurate at such levels; and (C) the reference substance must exhibit a BOD $\geq$60% of ThOD within a reasonably short time span (i.e., <10 days).

13.0 REPORT

A final report will be submitted to the Sponsor and will include, but not be limited to, the following:

- Study dates, name, and address of test facility.
- Objectives of the study.
- A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
- Description of test substance (e.g., date of receipt, storage conditions, purity, physical characteristics, and method of preparing stock and/or test solutions) and identification of the reference substance, if applicable.
- Description of seawater, including source, and date obtained.
- Results of the DO measurements.
- The calculated BOD and percent degradation. A plot of percent degradation versus time.
- Temperature range of the environmental chamber over the duration of the test.
- Summary of the data and a statement of the conclusions drawn from any data analyses, if appropriate.
- Description of any protocol deviations.
- Location of raw data.
- List of all technical personnel involved in the study and signature of the Study Director.
- GLP compliance statement by the Study Director and a statement by ABC Laboratories' Quality Assurance Unit.

14.0 PROTOCOL AMENDMENTS AND DEVIATIONS

The Study Director may make amendments to this protocol. All amendments will describe the change(s), the reason(s) for the amendment, and the effect on the study, if any, and will be communicated to the Sponsor Representative. All amendments will be signed and dated by at least the Study Director and maintained with the protocol.

In the event of a protocol deviation, a written description of the deviation including the reason for the deviation and any impact on the study as a result of the deviation will be communicated to the Sponsor Representative. All deviations will be signed and dated by at least the Study Director and maintained with the protocol.

15.0 QUALITY ASSURANCE

ABC's Quality Assurance Unit will inspect one or more critical phases to assure that equipment, personnel, procedures, and records conform to the guidelines listed in this protocol. The results of these inspections will be reported to the Study Director and ABC management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the Study Director and management.

16.0 GLP COMPLIANCE

All test procedures, documentation, records, and reports will comply with OECD Principles of Good Laboratory Practice (4). The report will contain a statement attesting to that fact.

17.0 RECORDS

Records to be maintained will include, but not be limited to, test substance receipt; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the study, all original raw data will be submitted to the Sponsor along with the final report. A copy of the final report, copies of all raw data from the study, and all original facility records will be kept on file in ABC Laboratories' archives.

18.0 SPECIMEN DISPOSAL

Following finalization of the report, disposition of all specimens (i.e., any material derived from the test system for examination, analysis, or retention) generated during the conduct of the test will be completed in a timely manner. Retention specimens holding time will be based on stability information provided by the Sponsor or by stability data generated by ABC

Laboratories. Retention specimens will be returned to the Sponsor unless archiving is contracted with ABC Laboratories. Documentation of specimen disposal will be retained with study records in ABC Laboratories' Archive.

19.0 REFERENCES

(1) Organization for Economic Cooperation and Development (OECD). July 17, 1992. OECD Guideline for the Testing of Chemicals. Biodegradability in Seawater, OECD Guideline No. 306.

(2) International Organization for Standardization (ISO). 1995. ISO Standard 10634: Water Quality — Guidance for the Preparation and Treatment of Poorly Water-Soluble Organic Compounds for the Subsequent Evaluation of Their Biodegradability in an Aqueous Medium.

(3) American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Part 9215 B. Pour Plate Method.

(4) Organization for Economic Cooperation and Development. 1997. Decision of the Council, Revised Principles of GLP [C(97)186/Final].

PROTOCOL APPROVAL

Study Director

Name (signed): Philip Sarff Date: 24 Jan 08

Name/Title: Philip Sarff / Senior Chemist

Sponsor Representative

Name (signed): Stephen P. Rabke Date: 1/28/08

Name/Title: Stephen P. Rabke / Manager – Occupational Health

Test Facility Management

Name (signed): Jeri Hofen Date: 24 JAN 2008

Name/Title: Jeri Hofen / Quality Assurance Associate II

QAU Protocol Review for GLP Compliance

Name (signed): Jon E. Rhodes Date: 24 Jan 08

Name/Title: Jon E. Rhodes / Director, ABC Chemical Services

ATTACHMENT 1

METHODS FOR INTRODUCING INSOLUBLE SUBSTANCES TO MEDIA
(Based on ISO Standard 10634)

- Prepare a solution (~ 500 mg C/L) of the test substance in an organic solvent (e.g., acetone or dichloromethane)
- For each test substance system: In a 250-mL round bottom flask, mix 30 g of silica gel with the volume of test substance solution required to provide the desired mass to test substance (i.e. for a 20 mg C/L solution, a total of 60 mg C is needed; if the test substance solution is 500 mg C/L, then 120 mL of solution is added). Dry the organic solvent using a rotary evaporator followed by a vacuum oven at 45°C. The entire dosed silica gel sample may be introduced into the system.
- For each control and reference substance system: Prepare untreated silica gel in the same manner using the same solvent (with no test substance) and volume.
- Removal of solvent can be verified by weighing the flask and silica gel before addition of solvent and after drying of solvent in the untreated silica gel samples.

Alternatively,

- Prepare a solution (~1000 mg/L) of the test substance in an organic solvent (e.g., acetone or dichloromethane)
- For test substance systems: In a 250-mL round bottom flask, mix 30 g of silica gel with 150 mL of the test substance solution. Dry the organic solvent using a rotary evaporator followed by a vacuum oven at 45°C. Using subsamples of the dosed silica gel, extract the test substance and analytically determine the amount of test substance adsorbed. The amount of silica gel to be introduced into the test systems may then be calculated. One batch of treated silica gel may be adequate for multiple systems.
- For control and reference substance system: Prepare untreated silica gel in the same manner using the same solvent (with no test substance) and volume. Introduce the same amount of untreated silica gel to the systems as was used for the test substance systems.
- Removal of solvent can be verified by weighing the flask and silica gel before addition of solvent and after drying of solvent in the untreated silica gel samples.

If a method for extracting the test substance from the silica gel is not available, then the first method must be used. In the first method a small portion of the test substance may coat the glass flask and cause the amount of test substance added to the system to be less than the nominal. The second method, therefore, would be more accurate since the amount of test substance coating the silica gel is analytically measured.

PROTOCOL AMENDMENT NOTIFICATION

PROTOCOL TITLE:	NOVAPLUS System: Determination of Biodegradability in Seawater Using the Closed Bottle Test Method
AMENDMENT NO.: 1	**EFFECTIVE DATE:** February 15, 2008
LABORATORY: ABC Laboratories, Inc.	**LAB STUDY NO.:** 63454
SPONSOR: M-I SWACO	

1. Protocol Sections 10.3 (Dissolved Oxygen and pH Measurements):
   The measurement of pH will only be performed at initiation and termination.

   The test will be terminated prior to Day 28. The exact day of termination will be reported in the study records and report.

   Reason: Measurement of pH is supplemental information, thus initiation and termination measurements are sufficient for pH characterization during the study. The test will be terminated early because the test and reference substances reached >60% biodegradation by Day 7 and confirmed by the results of Day 14.

   Effect on the Study: None.

STUDY DIRECTOR'S SIGNATURE:	P Philip Sanff	**DATE:** 18 Feb 2008
SPONSOR REPRESENTATIVE'S SIGNATURE:	Stephen P. Valle	**DATE:** 2/18/08
ABC MANAGEMENT SIGNATURE:	Jon E. Nalo	**DATE:** 18 Feb 08

PROTOCOL AMENDMENT NOTIFICATION

PROTOCOL TITLE:	NOVAPLUS System: Determination of Biodegradability in Seawater Using the Closed Bottle Test Method
AMENDMENT NO.: 2	**EFFECTIVE DATE:** February 28, 2008
LABORATORY: ABC Laboratories, Inc.	**LAB STUDY NO.:** 63454
SPONSOR: M-I SWACO	

1. Protocol:
   The title of the study is updated to:

   "NOVAPLUS System (Fluido Novaplus SBM com Novawet II): Determination of Biodegradability in Seawater Using the Closed Bottle Test Method."

   Reason: To include the Portuguese name of the test substance in the study title

   Effect on the Study: None.

STUDY DIRECTOR'S SIGNATURE:	Philip Smith	**DATE:** 28 Feb 2008
SPONSOR REPRESENTATIVE'S SIGNATURE:	Kirk D. Burke	**DATE:** 2/28/08
ABC MANAGEMENT SIGNATURE:	Jon E. Black	**DATE:** 28 Feb 08

PROTOCOL DEVIATION NOTIFICATION

STUDY TITLE:	NOVAPLUS System (Fluido Novaplus SBM com Novawet II): Determination of Biodegradability in Seawater Using the Closed Bottle Test Method
DEVIATION NO.: 1	**EFFECTIVE DATE:** February 29, 2008
LABORATORY: ABC Laboratories, Inc.	**LAB STUDY NO.:** 63454
SPONSOR: M-I SWACO	

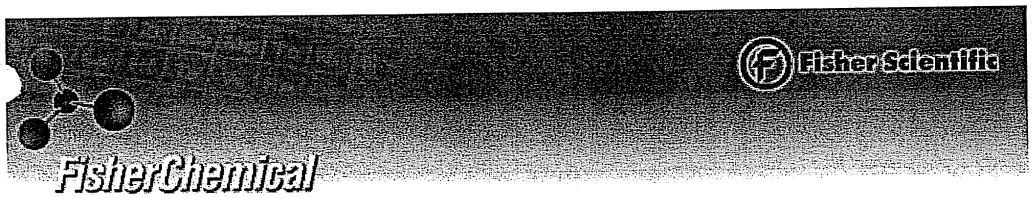
1. Protocol Section 10.1.1, Stock Solutions
   A 50.3065 g aliquot of $Na_2HPO_4 \cdot 7H_2O$ was used in the preparation of the phosphate buffer solution. This aliquot was not within $\pm$0.05 g of the desired molar equivalent of $Na_2HPO_4 \cdot 2H_2O$ (50.11 to 50.21 g).

   Reason: The amount weighed was that to make the molar equivalent of 33.40 g/L of $Na_2HPO_4 \cdot 2H_2O$, which is that used in the ready biodegradation method OECD 301D.

   Effect on the Study: None, as the resulting molarity was the same (0.19 M of the sodium phosphate dibasic).

STUDY DIRECTOR'S SIGNATURE:	Philip Swift	**DATE:** 03 Mar. 2008
SPONSOR REPRESENTATIVE'S SIGNATURE:	[illegible]	**DATE:** 3/3/08
ABC MANAGEMENT SIGNATURE:	[illegible]	**DATE:** 3 Mar 08

APPENDIX B – CERTIFICATES OF ANALYSIS



1 Reagent Lane
Fairlawn, NJ 07410
201.796.7100 tel
201.796.1329 fax

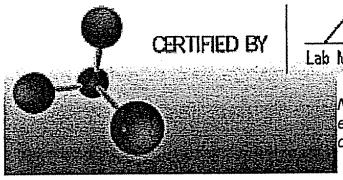
Certificate of Analysis

Fisher Scientific's Quality System is Certified to ISO9002 (1994) standard by DNV Cert. # 96-HOU-AQ-8052

This is to certify that units of the lot number mentioned below were tested and found to comply with the specifications of the grade listed. Certain data has been supplied by third parties. Fisher Scientific expressly disclaims all warranties, express or implied, including the implied warranties of merchantability and fitness for a particular purpose. Unless otherwise stated, these products are not intended for dialysis, parenteral or injectable use without further processing. The following are the actual analytical results obtained:

Catalog Number S224	**Mfg. Date** 05/04/2004
Lot Number 041447	**Sample ID** S224..041447.100
Description SODIUM BENZOATE NF/FCC	

Result Name	Units	Specifications	Test Value
ALKALINITY	PASS/FAIL	Pass test	PASS
APPEARANCE	REPORT	White granules or powder	WHITE POWDER
\SSAY	%	99.0 to 100.5	100.4000
ASSAY - FCC	%	99.0 - 100.5	100.4
HEAVY METALS(AS Pb)	%	0.001 Maximum	0.0003
HEAVY METALS(AS Pb)- FCC	mg/kg	10 Maximum	3.3
IDENTIFICATION	PASS/FAIL	Pass test	PASS
IDENTIFICATION - FCC	PASS/FAIL	Pass test	PASS
ORGANIC VOLATILE IMP		Meets requirements	PASS
WATER (FCC)	%	1.5 Maximum	0.2
WATER (H2O)	%	1.5 Maximum	0.300



Robert Dowd
Lab Manager Fair Lawn

Edgar E. Hess
Lab Manager BPF

Note: The data listed is valid for all package sizes of this lot of product, expressed as an extension of the catalog number listed above. If there are any questions with this certificate, please call Chemical Services at 1.800.227.6701.