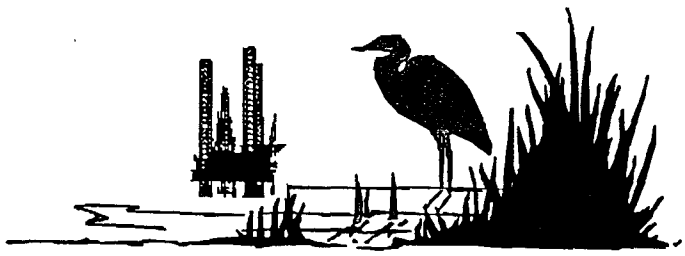

BIO-AQUATIC TESTING, INC.
ENVIRO-SCIENCES, INC.

BIOREMEDIATION EFFECTIVNESS TEST FOR

INTERNATIONAL ENVIRONMENTAL PRODUCTS

AGENT

Marc H. Bentley, M.S.
Aquatic Toxicologist

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EXECUTIVE SUMMARY

Bio-Aquatic Testing, Inc. located at 2501 Mayes Rd. Suite 100 Carrollton, Texas 75006 was contracted by International Environmental Products to test effectiveness of their bioremediation product using Environmental Protection Agency (EPA) protocol listed in 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, Item 4.0. The test protocol calls for application of products onto ANS 521 oil. The product was applied to test flasks according to manufacturer's specifications. Samples were sacrificed on Day 0, Day 7, and Day 28 of the test period. Day 0 and Day 7 samples were sampled for microbiological analysis and then frozen at –10 C until GC/MS results were known for the Day 28 samples. Each replicate of product and control were tested for continued microbiological viability over time, reduction in weight via gravimetric analysis, and reduction in alkane and/or aromatic constituents via Gas Chromatography/Mass Spectroscopy (GC/MS). The product was deemed effective if the data showed the GC/MS product results for Day 28 to be statistically less than the Day 28 controls at the end of the test period.

Microbiological results showed continued viability of the oil-eating microorganisms over time.

Statistical Analysis on gravimetric data showed the Day 28 product results to be significantly less than the Day 28 Control results.

GC/MS data for Days 0, 7, and 28, were consolidated and analyzed with Minitab Statistical program 13.3. The data was first analyzed for normality with the Anderson Darling test. If the data was deemed to have a normal distribution, it was analyzed for a significant difference between controls and treatments (products) using a general linear ANOVA model and Dunnett's test. If the data was not deemed to be normally distributed, it was first rank-transformed and then reanalyzed with the general linear ANOVA model and Dunnett's test. GC/MS analysis showed significant reduction of both alkane and aromatic constituents of the test oil as indicated by the statistically significant difference between the Day 28 controls and Day 28 treatments. The surrogate compounds, d-10 phenanthrene and 5-α androstane showed excellent recovery indicating the test to be valid.

Based on the parameters of this test, the product should seriously be considered for inclusion on the NCP list of approved bioremediation products.

BIOREMEDIATION AGENT EFFECTIVENESS TEST USING F5 S-200 NUTRIENT FORMULA

Introduction

The bioremediation agent effectiveness testing protocol is designed to determine a product's ability to biodegrade oil by quantifying changes in the oil composition resulting from biodegradation. The protocol quantifies the disappearance of saturated hydrocarbons and polynuclear aromatic hydrocarbons (PAHs) as well as weight loss. The protocol also tests for microbial activity over time to ascertain continued viability of oil degrading microorganisms.

Summary of Method

The protocol calls for gas chromatography/mass spectrophotometry and gravimetric analyses to quantify saturated hydrocarbons and PAHs, and determine weight loss, respectively. The sample preparation procedure calls for extracting the oil phase into dicloromethane (DCM), with a subsequent distillation to 1-3-mL using a K-D apparatus and Snyder column. Due to inconsistent surrogate recoveries experienced with distillation of the samples, the procedure was modified to substitute the distillation phase of the experiment with evaporation techniques accomplished with a Buchi Rotovapor apparatus.

The analytical technique uses a high-resolution gas chromatography/mass spectrophotometer (GC/MS) because of its high degree of chemical separation and spectral resolution. GC/MS has long been used to study the weathering and fate of oil spilled into the environment. For quantitative analyses, the instrument is operated in the selective ion detection mode (SIM) at a scan rate of greater than 1.5 scans per second to maximize the linear quantitative range and precision of the instrument. The sample preparation method does not exclude analysis of selected samples by GC/MS in the full scanning mode of operation to qualitatively assess changes in the oil not accounted for by the SIM approach.

Gravimetric analysis is used to support the GC/MS analysis by measuring weight loss of samples over time as compared to controls by drying the extracted samples using nitrogen a blowdown technique.

Microbiological analysis was performed concurrently with the chemical analysis. The analysis is performed to determine and monitor the viability of relative concentrations of the microbial cultures being studied. Continued viability is measured over time by comparing serial dilutions of microorganisms between treatments and controls, using this method.

MATERIALS AND METHODS

The following methods[1] were obtained from 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, item 4.0 Bioremediation Effectiveness test, as submitted by the Environmental Protection Agency. Some modifications were made to these methods as discussed below.

[1] Details from these methods can be found in the aforementioned 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, item 4.0. A copy is available upon request.

The procedure consists of an experimental orbital-shaker flask setup using 250-mL Erlenmeyer flasks labeled with unique identifiers using the following treatment design in Table 1.

Table 1. Experimental Design

Treatment	Number of samples at sampling times			Total number of analytical determinations		
	Day 0	Day 7	Day 28	ANALYSES		
				Microbial counts	Gravimetric	GC/MS
Control	3	3	3	9	9	9
F5 S-200	3	3	3	9	9	9

Number of replicates per treatment or control per sampling event - 3
Number of replicates per treatment or control – 9
Total replicates - 18
Control - Oil + Seawater
F5 S-200- Oil + Seawater + nutrient (F5 S-200 product)

Each appropriately labeled replicate flask has 100-mL of seawater added using aseptic technique. The seawater obtained was from the Gulf of Mexico and delivered via courier to Bio-Aquatic Testing, Inc., 2501 Mayes Rd., Carrollton, Texas 75006. Each flask is placed on a balance and the weight recorded. Approximately one half-gram (0.5 g) of artificially weathered oil (Alaska North Slope 521)[2] is then added to each flask while still on the balance and the weight recorded again.

The product/nutrient mix is prepared according to the manufacturer's instructions as follows. Information supplied with the product included an application rate of 10% of the hydrocarbon (oil). Consequently, approximately 0.05 g of product was added to each flask via micropipette with sterile tips, after the aliquots of oil were added. Nothing further was added to the control replicates. After preparing all treatments and controls, three replicates of each treatment and control were shaken on an orbital shaker at 190 – 200 rpm and incubated at 20° C until sacrificed for the Day 0, 7, and 28 analyses.

Sacrificed samples were then frozen at -10 C until sending them in a ice-chest packed with dry ice packets to the subcontracting laboratory.[3] The subcontracting laboratory, (care of Scott Miles), is Louisiana State University Institute for Environmental Studies (LSU-IES) for GC/MS analysis via overnight courier.

[2] The ANS 521 oil was obtained from John Haines of the Environmental Protection Agency's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268.

[3] The GC/MS analysis was subcontracted to Louisiana State University-IES, 42 Atkinson Hall, Baton Rouge, Louisiana, 70803.

Each replicate sacrificed was extracted with an initial volume of 50-mL dichloromethane (DCM) for the chemical analysis. The sample was first extracted three times with 10-mL aliquots of the DCM. The remaining 20-mL was used to rinse the separatory funnel and added to the first 30-mL of extract. Just prior to the initial extraction, each replicate is spiked with 1 mL of a 1000 µg/mL of a surrogate-recovery, standards stock solution. This stock solution was made up of 5α-androstane and d_{10}-phenanthrene.

The separatory funnel was then capped and shaken vigorously for approximately thirty seconds to insure good mixing between phases. After mixing, the separatory funnel was allowed to sit for up to three hours to insure the greatest amount of separation between phases. This was done because of the presence of thick emulsions caused by microbiological activity. After a period of up to 45 minutes, the oil phase was bled into a clean, 100-mL beaker. Each sample was then filtered through course filter paper containing 10 grams of sodium sulfate ($NaSO_4$). The sodium sulfate was dried in a 350° F oven overnight prior to use. After the initial filtration, spent sodium sulfate and filter paper were covered with an excess of DCM and subjected to sonication to mobilize any remaining oil. This solution was added to the initial extract and poured into a 300-mL boiling flask with etched necks. The boiling flask was then fixed onto the suction end of the Buchi Rotovapor apparatus and reduced to 3-4 mL. Reduced samples were stored in 40 mL amber vials with Teflon™ lined caps and secured with Teflon™ tape until analysis with GC/MS. After obtaining results, LSU corrected the raw data for percent surrogate recovery and entered the calculated results onto an MicroSoft Excel™ spreadsheet and electronically mailed to Marc Bentley, M.S., at Bio-Aquatic Testing, Inc. for statistical analysis and final write up.

STATISTICAL METHODS

GC/MS Data

Raw and surrogate adjusted data or rank transformed data were analyzed using the Minitab™ 13.3 program. The specific computer program used was a general linear ANOVA model with Dunnett's test capabilities. The probability of a type I error (α) was set apriori to 0.05.

Data sets were first analyzed for normality using the Anderson-Darling Goodness of Fit test. This test compares plot points with the normal theoretical distribution. Minitab calculates the statistic, above which there is a danger of non-normality. This is then compared to the chosen alpha (α) level of 0.01.

Routine transformations were not amenable to non-normal data so an acceptable procedure for multiple comparison ANOVA was found by rank-transforming the non-normal data (Helsel, et al, 1993). This technique first rank transforms the data and subjects it to the same multiple factor ANOVA and Dunnett's test used for normally distributed data. This makes the analysis an acceptable form of a single comparison non-parametric test. After the program calculated the "F" and "P" statistics, the data were automatically subjected to Dunnett's test for comparison between treatments and controls.

ANOVA tables below give the final adjusted P-Values. Values of less than 0.05 (chosen α) indicates statistical significance. The T-Value is a ratio of the Difference of Means and Standard Error of Difference and indicates the degree and direction of the difference. Dunnetts tables below indicate where the differences lie.

RESULTS AND DISCUSSION

GC/MS Data

Results of the statistical analysis for both raw and transformed data are reported and discussed below. Results for raw data are discussed first, and rank transformed data when applicable. Actual GC/MS data are presented in Appendix I. Computer printouts of the Minitab™ tests for normality, ANOVA, and Dunnett's test program appear in Appendix II.

Raw Alkane Data

Analysis of raw alkane data for normality (fig.1) showed the raw data to be normal with an Anderson-Darling P-statistic of 0.063, above the selected α-level of 0.01.

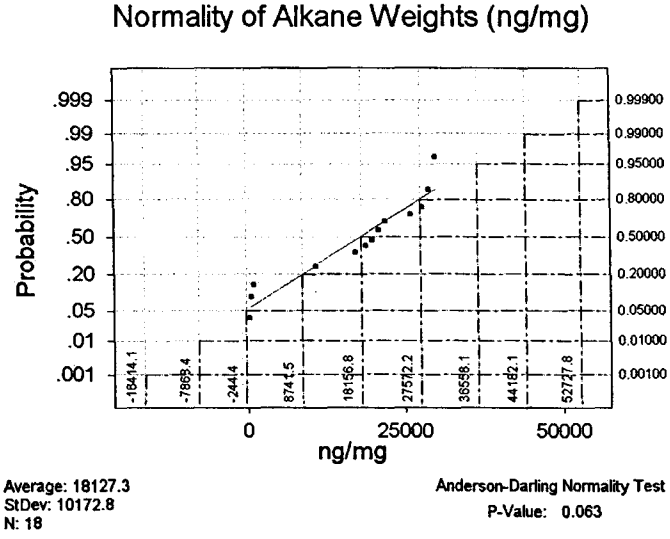


figure. 1 – Anderson-Darling test for normality showing linearity of raw alkane data.

Raw alkane data were analyzed with the multiple-comparison model ANOVA for normally distributed data and Dunnett's test using Day 0, 7, and 28 control for control levels (Table's 2, 3, 4, and 5). Table 2 P-statistics calculated by the ANOVA program for non-transformed treatments, and treatment/day interactions are all under the chosen alpha (α) level of 0.05 indicating a significant difference between one or more treatments for one or more days.

Table 2. ANOVA on raw alkane data

ANOVA non-transformed data						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	1	358512790	358512790	358512790	442.33	0.000
Day	2	1093189029	1093189029	546594515	674.38	0.000
Treatment*Day	2	297814914	297814914	148907457	183.72	0.000
Error	12	9726191	9726191	810516	----------	----------
Total	17	1759242924	----------	----------	----------	----------

The tables below show the results of statistical analysis using Dunnett's test for detecting the difference between a control level, other treatments and controls (as indicated by an adjusted P-value of less than the chosen alpha (α) level of 0.05). Table 3 shows P-values for Day 7 and 28 treatments and controls to be significantly less than Day 0 controls. Table 4 shows P-values for Day 7 and 28 treatments to be significantly less than Day 7 controls. Table 4 also shows Day 28 controls to be significantly less than Day 7 controls. Table 5 shows P-values for Day 7 and Day 28 treatments to be significantly less than Day 28 controls.

Table 3. Dunnett's test results using Day 0 controls as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	7	-6333	735.1	-8.92	0.0000
Control	28	-8896	735.1	-12.10	0.0000
F5 S-200	0	1667	735.1	2.27	0.9994
F5 S-200	7	-16667	735.1	-22.67	0.0000
F5 S-200	28	-27007	735.1	-36.74	0.0000

Table 4. Dunnett's test results using the Day 7 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	0	6333	735.1	8.62	1.0000
Control	28	-2563	735.1	-3.49	0.0089
F5 S-200	0	8000	735.1	10.88	1.0000
F5 S-200	7	-10333	735.1	-14.06	0.0000
F5 S-200	28	-20673	735.1	-28.12	0.0000

Table 5. Dunnett's test results using the Day 28 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	0	8896	735.1	12.10	1.0000
Control	7	2563	735.1	3.49	1.0000
F5 S-200	0	10563	735.1	14.37	1.0000
F5 S-200	7	-7771	735.1	-10.57	0.0000
F5 S-200	28	-18111	735.1	-24.64	0.0000

Raw Aromatic data

Preliminary analysis for the raw aromatic data (fig.2) for normality shows the data as non-linear with an Anderson-Darling P-statistic of 0.001. This is below the selected α-level of 0.01, and the data should be transformed before ANOVA analysis.

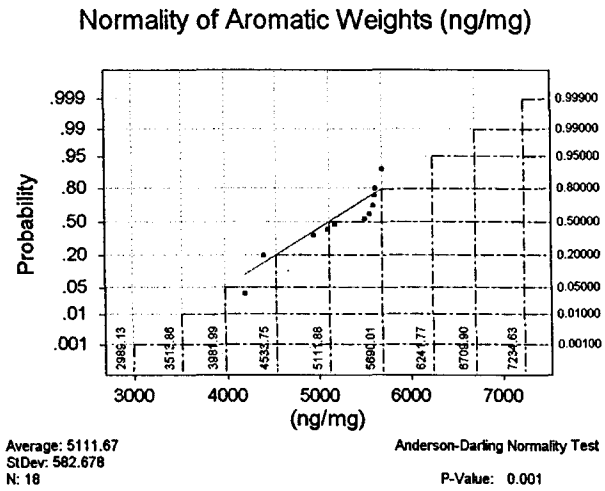


figure. 2 – Anderson-Darling test for normality showing non-linearity of the raw aromatic data.

Rank Transformed Raw Aromatic Data

Re-analysis for the raw aromatic data after transformation (fig. 3) for normality showed the data to have improved linearity with an Anderson-Darling P-statistic of 0.166. This is above the selected α-level of 0.01.

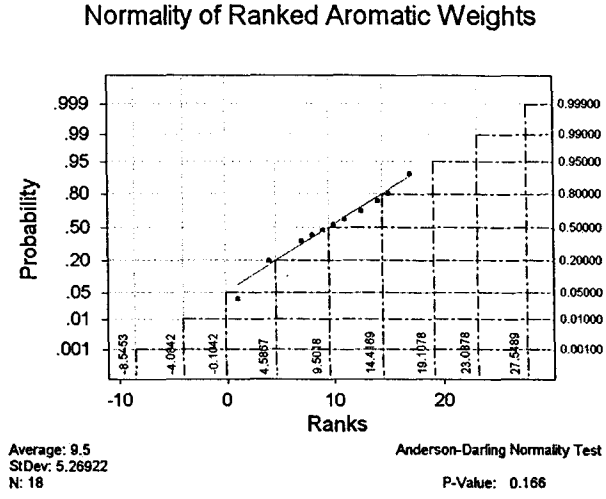


figure. 3 – Anderson-Darling test for normality showing improved linearity of transformed raw aromatic data.

Raw aromatic data were analyzed with ANOVA (Table 6) and Dunnett's test (Tables 7, 8, and 9) though the Anderson-Darling test demonstrated the data to be non-normal. Table 6 P-values for treatments and treatment/day interactions are all under the chosen alpha (α) level of 0.05 indicating a significant difference between one or more treatments for one or more days.

Table 6 ANOVA on raw aromatic data

ANOVA on transformed data						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	1	76310	76310	76310	10.86	0.006
Day	2	5262233	5262233	2631117	374.52	0.000
Treatment*Day	2	348887	348887	174444	24.83	0.000
Error	12	8435771734	84303	7025	----------	----------
Total	17	5771734	----------	----------	----------	----------

The tables below show the results of statistical analysis using Dunnett's test for detecting where the difference between a control level, other treatments and controls lie. This is indicated by an adjusted P-value of less than the chosen alpha (α) level of 0.05. Table 7 shows P-values for Day 7 and 28 treatments and Day 7 controls to be significantly less than Day 0 controls. P-values for Table 8 show no treatments or controls to be significantly less than Day 7 controls. Table 9 P-values show the Day 7 and 28 treatments as well as the Day 7 controls to be significantly less than the Day 28 controls.

Table 7 Dunnett's test results using the Day 0 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	7	-1267	68.44	-18.51	0.0000
Control	28	-3	68.44	-0.04	0.8202
F5 S-200	0	67	68.44	0.97	0.9803
F5 S-200	7	-1200	68.44	-17.53	0.0000
F5 S-200	28	-527	68.44	-7.70	0.0000

Table 8 Dunnett's test results using the Day 7 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	0	1266.67	68.44	18.5087	1.0000
Control	28	1263.67	68.44	18.4649	1.0000
F5 S-200	0	1333.33	68.44	19.4829	1.0000
F5 S-200	7	66.67	68.44	0.9741	0.9803
F5 S-200	28	739.67	68.44	10.8081	1.0000

Table 9 Dunnett's test results using the Day 28 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	0	3	68.44	0.04	0.8458
Control	7	-1264	68.44	-18.46	0.0000
F5 S-200	0	70	68.44	1.02	0.9824
F5 S-200	7	-1197	68.44	-17.49	0.0000
F5 S-200	28	-524	68.44	-7.66	0.0000

Raw aromatic data were then re-analyzed with ANOVA and Dunnett's test after rank-transformation (Table's 10, 11, 12, and 13). P-values for Day and Treatment/Day interactions are under the chosen alpha (α) level of 0.05. This indicates that at least two groups differ at some point of time.

Table 10 ANOVA on rank transformed aromatic data

ANOVA on transformed data

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	1	2.000	2.000	2.000	0.43	0.523
Day	2	364.333	364.333	182.167	39.51	0.000
Treatment*Day	2	50.333	50.333	25.167	5.46	0.021
Error	12	55.333	55.333	4.611	----------	----------
Total	17	472.000	----------	----------	----------	----------

The tables below show the results of statistical analysis using Dunnett's test for detecting the difference between a control level, other treatments and controls (as indicated by an adjusted P-value of less than the chosen alpha (α) level of 0.05). Table 11 shows P-values for Day 7 and 28 treatments as well as Day 7 controls to be significantly less than Day 0 controls. Table 12 P-values show no statistical difference between Day 7 controls and any other treatment or control. Table 13 P-values show Day 7 and 28 treatments as well as Day 7 controls to be significantly less than Day 28 controls.

Table 11 Dunnett's test results using the Day 0 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST

Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	7	-10.17	1.753	-5.799	0.0002
Control	28	0.17	1.753	0.095	0.8596
F5 S-200	0	2.33	1.753	1.331	0.9922
F5 S-200	7	-9.17	1.753	-5.228	0.0005
F5 S-200	28	-5.17	1.753	-2.947	0.0231

Table 12 Dunnett's test results using the Day 7 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	0	10.167	1.753	5.7986	1.0000
Control	28	10.333	1.753	5.8936	1.0000
F5 S-200	0	12.500	1.753	7.1294	1.0000
F5 S-200	7	1.000	1.753	0.5704	0.9477
F5 S-200	28	5.000	1.753	2.8518	0.9999

Table 13 Dunnett's test results using the Day 28 control as the control level vs. all other treatments and controls (all interactions).

DUNNETT'S TEST					
Treatment	Day	Difference of Means	Standard Error of Difference	T-Value	Adjusted P-Value
Control	0	-0.17	1.753	-0.095	0.8040
Control	7	-10.33	1.753	-5.894	0.0002
F5 S-200	0	2.17	1.753	1.236	0.9900
F5 S-200	7	-9.33	1.753	-5323	0.0004
F5 S-200	28	-5.33	1.753	-3.042	0.0196

MICROBIOLOGICAL DATA

Table 14 below shows the results showing the continued viability of microbial incubations over time. Most Probable Number (MPN) statistical analysis computer printouts can be seen in Appendix III.

Table 14 – Statistical Analysis of microbe viability

Treatments	**Day 0**	**Day 7**	**Day 28**
Control Rep# 1	**Cell Counts**	**Cell Counts**	**Cell Counts**
MPN	60.61	565.5	259.8
MPN corrected for bias	55.14	448.3	209.9
Spearman-Karber Estimate	100	1000	681.3
Control Rep #2			
MPN	00.00	401.8	00.00
MPN corrected for bias	00.00	302	00.00
Spearman-Karber Estimate	00.00	681.3	00.00
Control Rep #3			
MPN	36.1	1759	16.43
MPN corrected for bias	33.17	1507	15.2
Spearman-Karber Estimate	68.13	3162	46.42
Treatment Rep# 1			
MPN	60.61	485,236,187	TNTC*
MPN corrected for bias	55.14	346,842,093	TNTC*
Spearman-Karber Estimate	100	$.14678 \cdot E^{10}$	TNTC*
Treatment Rep# 2			
MPN	16.43	TNTC*	TNTC*
MPN corrected for bias	15.2	TNTC*	TNTC*
Spearman-Karber Estimate	46.42	TNTC*	TNTC*
Treatment Rep# 3			
MPN	141.7	TNTC*	TNTC*
MPN corrected for bias	123.4	TNTC*	TNTC*
Spearman-Karber Estimate	215.4	TNTC*	TNTC*

* - TNTC –Too Numerous to Count

Gravimetric Data

The following tables show the P-Values calculated by the two-sample t-test of the Minitab™ program. Table 15 shows calculated values for Day 28 controls and the product is lower than the chosen alpha (α) level of 0.05 and therefore indicate statistical significance. A computer printout of the analyses can be seen in Appendix IV.

Table 15. P-Values calculated by the two-sample t-test for product (F5 S-200) and controls

Treatments	Treatment Weights and Means (mg)				T-test Scores (p values)
	Rep#1	Rep#2	Rep#3	Mean	
Controls Day 0	0.130	0.140	0.150	0.140	
Product Day 0	0.14	0.130	0.130	0.133	**0.391**
Controls Day 7	0.130	0.120	0.120	0.123	
Product Day 7	0.100	0.090	0.120	0.103	**0.168**
Controls Day 28	0.140	0.180	0.180	0.166	
Products Day 28	0.100	0.080	0.110	0.096	**0.022**

Conclusions

Analysis of the raw, non-transformed treated alkane data shows sufficient degradation over time as compared to all controls for the product. There was statistically significant degradation of many treatments as compared to the Day 0 and Day 7 controls. Most importantly, the final comparison between Day 28 controls and treatments showed statistically significant degradation.

GC/ MS results are in turn supported by results seen in microbiological phase of the test. Results showed strong viability in the treatments as opposed to the controls even at Day 28.

Gravimetric statistical analysis also showed the product weights significantly less than the controls on Day 28.

All parameters tested showed clear evidence of product efficacy. Based upon the parameters of this test, the product should seriously be considered for inclusion to the NCP list of approved products.

GC/MS DATA

DES/RCAT Results for: Bio-Aquatic Testing, Inc
30 May 2002

CONTROL REPLICATE 1		
Testing Date: Day 0		
Initial Oil Weight: 510 mg		
Final Extracted Volume: 10.0 mL		
	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	27	27
nC-11 Undecane	26	26
nC-12 Dodecane	13	13
nC-13 Tridecane	8.8	8.8
nC-14 Tetradecane	170	170
nC-15 Pentadecane	1400	1400
nC-16 Hexadecane	2000	2000
nC-17 Heptadecane	1900	1900
Pristane	1200	1200
nC-18 Octadecane	1600	1600
Phytane	960	960
nC-19 Nonadecane	1800	1800
nC-20 Eicosane	1600	1600
nC-21 Heneicosane	1600	1600
nC-22 Docosane	1600	1600
nC-23 Tricosane	1700	1700
nC-24 Tetracosane	1400	1400
nC-25 Pentacosane	1400	1400
nC-26 Hexacosane	1400	1400
nC-27 Heptacosane	1100	1100
nC-28 Octacosane	1100	1100
nC-29 Nonacosane	820	820
nC-30 Triacontane	770	770
nC-31 Hentriacontane	740	740
nC-32 Dotriacontane	590	590
nC-33 Tritriacontane	550	550
nC-34 Tetratriacontane	330	330
nC-35 Pentatriacontane	390	390
Total Alkanes	28000	28000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	1.9	1.9
C1-Naphthalenes	6.7	6.7
C2-Naphthalenes	250	250
C3-Naphthalenes	760	760
C4-Naphthalenes	510	510
Fluorene	63	63
C1-Fluorenes	180	180
C2-Fluorenes	180	180
C3- Fluorenes	190	190
Dibenzothiophene	180	180
C1-Dibenzothiophenes	270	270
C2-Dibenzothiophenes	310	310
C3- Dibenzothiophenes	200	200
Phenanthrene	200	200
C1-Phenanthrenes	380	380
C2-Phenanthrenes	390	390
C3-Phenanthrenes	280	280
C4-Phenanthrenes	110	110
Anthracene	0.38	0.38
Fluoranthene	4.7	4.7
Pyrene	2.6	2.6
C1- Pyrenes	59	59
C2- Pyrenes	90	90
C3- Pyrenes	93	93
C4- Pyrenes	64	64
Naphthobenzothiophene	45	45
C-1 Naphthobenzothiophenes	160	160
C-2 Naphthobenzothiophenes	170	170
C-3 Naphthobenzothiophenes	120	120
Benzo (a) Anthracene	4.8	4.8
Chrysene	46	46
C1- Chrysenes	76	76
C2- Chrysenes	100	100
C3- Chrysenes	83	83
C4- Chrysenes	56	56
Benzo (b) Fluoranthene	1.9	1.9
Benzo (k) Fluoranthene	1.9	1.9
Benzo (e) Pyrene	1.3	1.3
Benzo (a) Pyrene	1.0	1.0
Perylene	0.35	0.35
Indeno (1,2,3 - cd) Pyrene	0.16	0.16
Dibenzo (a,h) anthracene	0.28	0.28
Benzo (g,h,i) perylene	0.46	0.46
Total Aromatics	5600	5600
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00

DES/RCAT Results for: Bio-Aquatic Testing, Inc
30 May 2002

CONTROL REPLICATE 2		
Testing Date: Day 0		
Initial Oil Weight: 500 mg		
Final Extracted Volume: 10.0 mL		
	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	22	22
nC-11 Undecane	26	26
nC-12 Dodecane	14	14
nC-13 Tridecane	8.6	8.6
nC-14 Tetradecane	170	170
nC-15 Pentadecane	1400	1400
nC-16 Hexadecane	2100	2100
nC-17 Heptadecane	2000	2000
Pristane	1200	1200
nC-18 Octadecane	1625	1625
Phytane	1100	1100
nC-19 Nonadecane	1800	1800
nC-20 Eicosane	1700	1700
nC-21 Heneicosane	1700	1700
nC-22 Docosane	1600	1600
nC-23 Tricosane	1600	1600
nC-24 Tetracosane	1400	1400
nC-25 Pentacosane	1400	1400
nC-26 Hexacosane	1400	1400
nC-27 Heptacosane	1200	1200
nC-28 Octacosane	990	990
nC-29 Nonacosane	790	790
nC-30 Triacontane	720	720
nC-31 Hentriacontane	730	730
nC-32 Dotriacontane	560	560
nC-33 Tritriacontane	540	540
nC-34 Tetratriacontane	360	360
nC-35 Pentatriacontane	430	430
Total Alkanes	29000	29000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	2.0	2.0
C1-Naphthalenes	7.1	7.1
C2-Naphthalenes	260	260
C3-Naphthalenes	780	780
C4-Naphthalenes	520	520
Fluorene	68	68
C1-Fluorenes	180	180
C2-Fluorenes	180	180
C3- Fluorenes	190	190
Dibenzothiophene	190	190
C1-Dibenzothiophenes	280	280
C2-Dibenzothiophenes	310	310
C3- Dibenzothiophenes	200	200
Phenanthrene	200	200
C1-Phenanthrenes	380	380
C2-Phenanthrenes	390	390
C3-Phenanthrenes	280	280
C4-Phenanthrenes	110	110
Anthracene	0.41	0.41
Fluoranthene	4.8	4.8
Pyrene	2.1	2.1
C1- Pyrenes	65	65
C2- Pyrenes	91	91
C3- Pyrenes	97	97
C4- Pyrenes	68	68
Naphthobenzothiophene	44	44
C-1 Naphthobenzothiophenes	160	160
C-2 Naphthobenzothiophenes	160	160
C-3 Naphthobenzothiophenes	120	120
Benzo (a) Anthracene	5.3	5.3
Chrysene	48	48
C1- Chrysenes	70	70
C2- Chrysenes	100	100
C3- Chrysenes	85	85
C4- Chrysenes	61	61
Benzo (b) Fluoranthene	1.9	1.9
Benzo (k) Fluoranthene	1.9	1.9
Benzo (e) Pyrene	1.5	1.5
Benzo (a) Pyrene	1.1	1.1
Perylene	0.34	0.34
Indeno (1,2,3 - cd) Pyrene	0.15	0.15
Dibenzo (a,h) anthracene	0.25	0.25
Benzo (g,h,i) perylene	0.47	0.47
Total Aromatics	5700	5700
Phenanthrene d-10	2000	2000
% Surrogate Recovery		

DES/RCAT Results for: Bio-Aquatic Testing, Inc
30 May 2002

CONTROL REPLICATE 3		
Testing Date: Day 0		
Initial Oil Weight: 510 mg		
Final Extracted Volume: 10.0 mL		
	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	23	23
nC-11 Undecane	25	25
nC-12 Dodecane	13	13
nC-13 Tridecane	8.6	8.6
nC-14 Tetradecane	170	170
nC-15 Pentadecane	1300	1300
nC-16 Hexadecane	1900	1900
nC-17 Heptadecane	1700	1700
Pristane	1200	1200
nC-18 Octadecane	1500	1500
Phytane	960	960
nC-19 Nonadecane	1600	1600
nC-20 Eicosane	1500	1500
nC-21 Heneicosane	1500	1500
nC-22 Docosane	1400	1400
nC-23 Tricosane	1400	1400
nC-24 Tetracosane	1300	1300
nC-25 Pentacosane	1300	1300
nC-26 Hexacosane	1300	1300
nC-27 Heptacosane	1100	1100
nC-28 Octacosane	910	910
nC-29 Nonacosane	730	730
nC-30 Triacontane	690	690
nC-31 Hentriacontane	660	660
nC-32 Dotriacontane	500	500
nC-33 Tritriacontane	470	470
nC-34 Tetratriacontane	340	340
nC-35 Pentatriacontane	420	420
Total Alkanes	26000	26000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	2.0	2.0
C1-Naphthalenes	6.5	7
C2-Naphthalenes	240	240
C3-Naphthalenes	710	710
C4-Naphthalenes	500	500
Fluorene	62	62
C1-Fluorenes	180	180
C2-Fluorenes	180	180
C3- Fluorenes	180	180
Dibenzothiophene	170	170
C1-Dibenzothiophenes	260	260
C2-Dibenzothiophenes	310	310
C3- Dibenzothiophenes	200	200
Phenanthrene	190	190
C1-Phenanthrenes	370	370
C2-Phenanthrenes	380	380
C3-Phenanthrenes	260	260
C4-Phenanthrenes	100	100
Anthracene	0.43	0.43
Fluoranthene	4.8	4.8
Pyrene	2.4	2.4
C1- Pyrenes	62	62
C2- Pyrenes	95	95
C3- Pyrenes	99	99
C4- Pyrenes	63	63
Naphthobenzothiophene	43	43
C-1 Naphthobenzothiophenes	160	160
C-2 Naphthobenzothiophenes	160	160
C-3 Naphthobenzothiophenes	130	130
Benzo (a) Anthracene	4.9	4.9
Chrysene	45	45
C1- Chrysenes	75	75
C2- Chrysenes	96	96
C3- Chrysenes	81	81
C4- Chrysenes	61	61
Benzo (b) Fluoranthene	1.9	1.9
Benzo (k) Fluoranthene	1.9	1.9
Benzo (e) Pyrene	1.5	1.5
Benzo (a) Pyrene	1.0	1.0
Perylene	0.39	0.39
Indeno (1,2,3 - cd) Pyrene	0.17	0.17
Dibenzo (a,h) anthracene	0.28	0.28
Benzo (g,h,i) perylene	0.48	0.48
Total Aromatics	5500	5500
Phenanthrene d-10	2000	2000
% Surrogate Recovery		

DES/RCAT Results for: Bio-Aquatic Testing, Inc
30 May 2002

TREATMENT REPLICATE 1		
	Testing Date: Day 0	
Initial Oil Weight: 520 mg		
Final Extracted Volume: 10.1 mL		
	Concentration (ng/mg)	**Surrogate Corrected (ng/mg)**
Alkane Analyte:		
nC-10 Decane	11	11
nC-11 Undecane	22	22
nC-12 Dodecane	130	130
nC-13 Tridecane	6.8	6.8
nC-14 Tetradecane	130	130
nC-15 Pentadecane	1400	1400
nC-16 Hexadecane	2200	2200
nC-17 Heptadecane	2100	2100
Pristane	1300	1300
nC-18 Octadecane	1800	1800
Phytane	1000	1000
nC-19 Nonadecane	2100	2100
nC-20 Eicosane	1900	1900
nC-21 Heneicosane	1800	1800
nC-22 Docosane	1700	1700
nC-23 Tricosane	1700	1700
nC-24 Tetracosane	1300	1300
nC-25 Pentacosane	1300	1300
nC-26 Hexacosane	1300	1300
nC-27 Heptacosane	1000	1000
nC-28 Octacosane	930	930
nC-29 Nonacosane	800	800
nC-30 Triacontane	720	720
nC-31 Hentriacontane	660	660
nC-32 Dotriacontane	500	500
nC-33 Tritriacontane	420	420
nC-34 Tetratriacontane	370	370
nC-35 Pentatriacontane	450	450
Total Alkanes	29000	29000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	1.7	1.7
C1-Naphthalenes	6.3	6.3
C2-Naphthalenes	220	220
C3-Naphthalenes	690	690
C4-Naphthalenes	450	450
Fluorene	65	65
C1-Fluorenes	190	190
C2-Fluorenes	190	190
C3- Fluorenes	200	200
Dibenzothiophene	190	190
C1-Dibenzothiophenes	300	300
C2-Dibenzothiophenes	330	330
C3- Dibenzothiophenes	210	210
Phenanthrene	230	230
C1-Phenanthrenes	420	420
C2-Phenanthrenes	440	440
C3-Phenanthrenes	320	320
C4-Phenanthrenes	120	120
Anthracene	0.36	0.36
Fluoranthene	5.4	5.4
Pyrene	2.3	2.3
C1- Pyrenes	60	60
C2- Pyrenes	89	89
C3- Pyrenes	94	94
C4- Pyrenes	61	61
Naphthobenzothiophene	40	40
C-1 Naphthobenzothiophenes	150	150
C-2 Naphthobenzothiophenes	150	150
C-3 Naphthobenzothiophenes	120	120
Benzo (a) Anthracene	4.6	4.6
Chrysene	44	44
C1- Chrysenes	67	67
C2- Chrysenes	98	98
C3- Chrysenes	75	75
C4- Chrysenes	48	48
Benzo (b) Fluoranthene	1.9	1.9
Benzo (k) Fluoranthene	1.9	1.9
Benzo (e) Pyrene	1.2	1.2
Benzo (a) Pyrene	0.84	0.84
Perylene	0.34	0.34
Indeno (1,2,3 - cd) Pyrene	0.16	0.16
Dibenzo (a,h) anthracene	0.26	0.26
Benzo (g,h,i) perylene	0.48	0.48
Total Aromatics	5700	5700
Phenanthrene d-10	2000	2000
% Surrogate Recovery		

DES/RCAT Results for: Bio-Aquatic Testing, Inc
30 May 2002

TREATMENT REPLICATE 2		
Testing Date: Day 0		
Initial Oil Weight: 500 mg		
Final Extracted Volume: 10.0 mL		
	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	12	12
nC-11 Undecane	25	25
nC-12 Dodecane	140	140
nC-13 Tridecane	7.1	7.1
nC-14 Tetradecane	140	140
nC-15 Pentadecane	1500	1500
nC-16 Hexadecane	2300	2300
nC-17 Heptadecane	2000	2000
Pristane	1300	1300
nC-18 Octadecane	1700	1700
Phytane	1100	1100
nC-19 Nonadecane	1900	1900
nC-20 Eicosane	1900	1900
nC-21 Heneicosane	1700	1700
nC-22 Docosane	1500	1500
nC-23 Tricosane	1700	1700
nC-24 Tetracosane	1500	1500
nC-25 Pentacosane	1400	1400
nC-26 Hexacosane	1400	1400
nC-27 Heptacosane	1200	1200
nC-28 Octacosane	1000	1000
nC-29 Nonacosane	770	770
nC-30 Triacontane	770	770
nC-31 Hentriacontane	710	710
nC-32 Dotriacontane	540	540
nC-33 Tritriacontane	470	470
nC-34 Tetratriacontane	390	390
nC-35 Pentatriacontane	450	450
Total Alkanes	30000	30000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	1.9	1.9
C1-Naphthalenes	6.1	6.1
C2-Naphthalenes	240	240
C3-Naphthalenes	720	720
C4-Naphthalenes	480	480
Fluorene	62	62
C1-Fluorenes	200	200
C2-Fluorenes	200	200
C3- Fluorenes	190	190
Dibenzothiophene	200	200
C1-Dibenzothiophenes	290	290
C2-Dibenzothiophenes	310	310
C3- Dibenzothiophenes	200	200
Phenanthrene	230	230
C1-Phenanthrenes	390	390
C2-Phenanthrenes	410	410
C3-Phenanthrenes	300	300
C4-Phenanthrenes	91	91
Anthracene	0.30	0.30
Fluoranthene	4.9	4.9
Pyrene	2.2	2.2
C1- Pyrenes	63	63
C2- Pyrenes	92	92
C3- Pyrenes	100	100
C4- Pyrenes	64	64
Naphthobenzothiophene	42	42
C-1 Naphthobenzothiophenes	160	160
C-2 Naphthobenzothiophenes	160	160
C-3 Naphthobenzothiophenes	120	120
Benzo (a) Anthracene	4.5	4.5
Chrysene	46	46
C1- Chrysenes	67	67
C2- Chrysenes	98	98
C3- Chrysenes	78	78
C4- Chrysenes	50	50
Benzo (b) Fluoranthene	2.0	2.0
Benzo (k) Fluoranthene	2.0	2.0
Benzo (e) Pyrene	1.3	1.3
Benzo (a) Pyrene	0.80	0.80
Perylene	0.26	0.26
Indeno (1,2,3 - cd) Pyrene	0.19	0.19
Dibenzo (a,h) anthracene	0.25	0.25
Benzo (g,h,i) perylene	0.40	0.40
Total Aromatics	5700	5700
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00

DES/RCAT Results for: Bio-Aquatic Testing, Inc
30 May 2002

TREATMENT REPLICATE 3		
Testing Date: Day 0		
Initial Oil Weight: 500 mg		
Final Extracted Volume: 10.1 mL		
	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	12	12
nC-11 Undecane	24	24
nC-12 Dodecane	150	150
nC-13 Tridecane	9.2	9.2
nC-14 Tetradecane	160	160
nC-15 Pentadecane	1300	1300
nC-16 Hexadecane	2100	2100
nC-17 Heptadecane	1800	1800
Pristane	1200	1200
nC-18 Octadecane	1600	1600
Phytane	1000	1000
nC-19 Nonadecane	1700	1700
nC-20 Eicosane	1800	1800
nC-21 Heneicosane	1700	1700
nC-22 Docosane	1600	1600
nC-23 Tricosane	1600	1600
nC-24 Tetracosane	1400	1400
nC-25 Pentacosane	1400	1400
nC-26 Hexacosane	1300	1300
nC-27 Heptacosane	1200	1200
nC-28 Octacosane	1100	1100
nC-29 Nonacosane	810	810
nC-30 Triacontane	830	830
nC-31 Hentriacontane	760	760
nC-32 Dotriacontane	560	560
nC-33 Tritriacontane	510	510
nC-34 Tetratriacontane	390	390
nC-35 Pentatriacontane	480	480
Total Alkanes	29000	29000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	1.8	1.8
C1-Naphthalenes	6.1	6.1
C2-Naphthalenes	230	230
C3-Naphthalenes	750	750
C4-Naphthalenes	480	480
Fluorene	63	63
C1-Fluorenes	190	190
C2-Fluorenes	180	180
C3- Fluorenes	180	180
Dibenzothiophene	180	180
C1-Dibenzothiophenes	270	270
C2-Dibenzothiophenes	310	310
C3- Dibenzothiophenes	200	200
Phenanthrene	200	200
C1-Phenanthrenes	390	390
C2-Phenanthrenes	410	410
C3-Phenanthrenes	290	290
C4-Phenanthrenes	100	100
Anthracene	0.39	0.39
Fluoranthene	4.5	4.5
Pyrene	2.7	2.7
C1- Pyrenes	62	62
C2- Pyrenes	85	85
C3- Pyrenes	97	97
C4- Pyrenes	60	60
Naphthobenzothiophene	42	42
C-1 Naphthobenzothiophenes	160	160
C-2 Naphthobenzothiophenes	160	160
C-3 Naphthobenzothiophenes	120	120
Benzo (a) Anthracene	4.7	4.7
Chrysene	46	46
C1- Chrysenes	70	70
C2- Chrysenes	95	95
C3- Chrysenes	78	78
C4- Chrysenes	57	57
Benzo (b) Fluoranthene	2.2	2.2
Benzo (k) Fluoranthene	1.6	1.6
Benzo (e) Pyrene	1.2	1.2
Benzo (a) Pyrene	0.73	0.73
Perylene	0.32	0.32
Indeno (1,2,3 - cd) Pyrene	0.14	0.14
Dibenzo (a,h) anthracene	0.24	0.24
Benzo (g,h,i) perylene	0.45	0.45
Total Aromatics	5600	5600
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00

CONTROL REPLICATE 1
Testing Date: Day 7
Initial Oil Weight: 500 mg
Final Extracted Volume: 10.1 mL

	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	9.3	9.3
nC-11 Undecane	17	17
nC-12 Dodecane	9.4	9.4
nC-13 Tridecane	5.8	5.8
nC-14 Tetradecane	110	110
nC-15 Pentadecane	900	900
nC-16 Hexadecane	1400	1400
nC-17 Heptadecane	1400	1400
Pristane	960	960
nC-18 Octadecane	1200	1200
Phytane	780	780
nC-19 Nonadecane	1300	1300
nC-20 Eicosane	1100	1100
nC-21 Heneicosane	1200	1200
nC-22 Docosane	1200	1200
nC-23 Tricosane	1200	1200
nC-24 Tetracosane	1200	1200
nC-25 Pentacosane	1100	1100
nC-26 Hexacosane	1200	1200
nC-27 Heptacosane	880	880
nC-28 Octacosane	780	780
nC-29 Nonacosane	550	550
nC-30 Triacontane	500	500
nC-31 Hentriacontane	470	470
nC-32 Dotriacontane	410	410
nC-33 Tritriacontane	400	400
nC-34 Tetratriacontane	230	230
nC-35 Pentatriacontane	280	280
Total Alkanes	21000	21000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	0.83	0.83
C1-Naphthalenes	2.9	2.9
C2-Naphthalenes	140	140
C3-Naphthalenes	590	590
C4-Naphthalenes	380	380
Fluorene	43	43
C1-Fluorenes	150	150
C2-Fluorenes	160	160
C3- Fluorenes	150	150
Dibenzothiophene	150	150
C1-Dibenzothiophenes	200	200
C2-Dibenzothiophenes	230	230
C3- Dibenzothiophenes	150	150
Phenanthrene	150	150
C1-Phenanthrenes	320	320
C2-Phenanthrenes	310	310
C3-Phenanthrenes	180	180
C4-Phenanthrenes	90	90
Anthracene	0.18	0.18
Fluoranthene	3.9	3.9
Pyrene	3.1	3.1
C1- Pyrenes	57	57
C2- Pyrenes	78	78
C3- Pyrenes	86	86
C4- Pyrenes	59	59
Naphthobenzothiophene	33	33
C-1 Naphthobenzothiophenes	120	120
C-2 Naphthobenzothiophenes	140	140
C-3 Naphthobenzothiophenes	110	110
Benzo (a) Anthracene	2.8	2.8
Chrysene	42	42
C1- Chrysenes	69	69
C2- Chrysenes	88	88
C3- Chrysenes	73	73
C4- Chrysenes	53	53
Benzo (b) Fluoranthene	1.3	1.3
Benzo (k) Fluoranthene	1.2	1.2
Benzo (e) Pyrene	1.3	1.3
Benzo (a) Pyrene	0.51	0.51
Perylene	0.24	0.24
Indeno (1,2,3 - cd) Pyrene	0.12	0.12
Dibenzo (a,h) anthracene	0.08	0.08
Benzo (g,h,i) perylene	0.16	0.16
Total Aromatics	4400	4400
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00
Phenanthrene d-10	1.00	1.00

CONTROL REPLICATE 2
Testing Date: Day 7
Initial Oil Weight: 520 mg
Final Extracted Volume: 10.0 mL

	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	9.3	9.3
nC-11 Undecane	16	16
nC-12 Dodecane	9.2	9.2
nC-13 Tridecane	5.4	5.4
nC-14 Tetradecane	110	110
nC-15 Pentadecane	860	860
nC-16 Hexadecane	1400	1400
nC-17 Heptadecane	1400	1400
Pristane	920	920
nC-18 Octadecane	1100	1100
Phytane	730	730
nC-19 Nonadecane	1200	1200
nC-20 Eicosane	1100	1100
nC-21 Heneicosane	1100	1100
nC-22 Docosane	1100	1100
nC-23 Tricosane	1100	1100
nC-24 Tetracosane	1300	1300
nC-25 Pentacosane	1200	1200
nC-26 Hexacosane	1200	1200
nC-27 Heptacosane	1000	1000
nC-28 Octacosane	910	910
nC-29 Nonacosane	560	560
nC-30 Triacontane	590	590
nC-31 Hentriacontane	540	540
nC-32 Dotriacontane	440	440
nC-33 Tritriacontane	410	410
nC-34 Tetratriacontane	230	230
nC-35 Pentatriacontane	280	280
Total Alkanes	21000	21000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	0.84	0.84
C1-Naphthalenes	3.3	3.3
C2-Naphthalenes	167	167
C3-Naphthalenes	600	600
C4-Naphthalenes	390	390
Fluorene	41	41
C1-Fluorenes	140	140
C2-Fluorenes	150	150
C3- Fluorenes	140	140
Dibenzothiophene	140	140
C1-Dibenzothiophenes	200	200
C2-Dibenzothiophenes	230	230
C3- Dibenzothiophenes	150	150
Phenanthrene	170	170
C1-Phenanthrenes	300	300
C2-Phenanthrenes	310	310
C3-Phenanthrenes	170	170
C4-Phenanthrenes	84	84
Anthracene	0.17	0.17
Fluoranthene	3.8	3.8
Pyrene	3.0	3.0
C1- Pyrenes	66	66
C2- Pyrenes	85	85
C3- Pyrenes	81	81
C4- Pyrenes	61	61
Naphthobenzothiophene	33	33
C-1 Naphthobenzothiophenes	130	130
C-2 Naphthobenzothiophenes	140	140
C-3 Naphthobenzothiophenes	100	100
Benzo (a) Anthracene	2.6	2.6
Chrysene	43	43
C1- Chrysenes	68	68
C2- Chrysenes	85	85
C3- Chrysenes	69	69
C4- Chrysenes	52	52
Benzo (b) Fluoranthene	1.2	1.2
Benzo (k) Fluoranthene	1.2	1.2
Benzo (e) Pyrene	1.3	1.3
Benzo (a) Pyrene	0.47	0.47
Perylene	0.25	0.25
Indeno (1,2,3 - cd) Pyrene	0.12	0.12
Dibenzo (a,h) anthracene	0.08	0.08
Benzo (g,h,i) perylene	0.16	0.16
Total Aromatics	4400	4400
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00
Phenanthrene d-10	1.00	1.00

CONTROL REPLICATE 3
Testing Date: Day 7
Initial Oil Weight: 510 mg
Final Extracted Volume: 10.0 mL

	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	9.2	9.2
nC-11 Undecane	16	16
nC-12 Dodecane	9.0	9.0
nC-13 Tridecane	5.6	5.6
nC-14 Tetradecane	110	110
nC-15 Pentadecane	890	890
nC-16 Hexadecane	1400	1400
nC-17 Heptadecane	1400	1400
Pristane	920	920
nC-18 Octadecane	1200	1200
Phytane	780	780
nC-19 Nonadecane	1300	1300
nC-20 Eicosane	1200	1200
nC-21 Heneicosane	1200	1200
nC-22 Docosane	1200	1200
nC-23 Tricosane	1200	1200
nC-24 Tetracosane	1200	1200
nC-25 Pentacosane	1200	1200
nC-26 Hexacosane	1200	1200
nC-27 Heptacosane	1000	1000
nC-28 Octacosane	890	890
nC-29 Nonacosane	660	660
nC-30 Triacontane	650	650
nC-31 Hentriacontane	610	610
nC-32 Dotriacontane	450	450
nC-33 Tritriacontane	390	390
nC-34 Tetratriacontane	220	220
nC-35 Pentatriacontane	240	240
Total Alkanes	22000	22000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	0.82	0.82
C1-Naphthalenes	2.5	2.5
C2-Naphthalenes	130	130
C3-Naphthalenes	450	450
C4-Naphthalenes	390	390
Fluorene	34	34
C1-Fluorenes	140	140
C2-Fluorenes	160	160
C3- Fluorenes	150	150
Dibenzothiophene	130	130
C1-Dibenzothiophenes	200	200
C2-Dibenzothiophenes	240	240
C3- Dibenzothiophenes	160	160
Phenanthrene	110	110
C1-Phenanthrenes	280	280
C2-Phenanthrenes	330	330
C3-Phenanthrenes	190	190
C4-Phenanthrenes	87	87
Anthracene	0.18	0.18
Fluoranthene	3.6	3.6
Pyrene	3.0	3.0
C1- Pyrenes	58	58
C2- Pyrenes	77	77
C3- Pyrenes	82	82
C4- Pyrenes	59	59
Naphthobenzothiophene	31	31
C-1 Naphthobenzothiophenes	120	120
C-2 Naphthobenzothiophenes	140	140
C-3 Naphthobenzothiophenes	110	110
Benzo (a) Anthracene	2.4	2.4
Chrysene	41	41
C1- Chrysenes	67	67
C2- Chrysenes	86	86
C3- Chrysenes	68	68
C4- Chrysenes	52	52
Benzo (b) Fluoranthene	1.2	1.2
Benzo (k) Fluoranthene	1.1	1.1
Benzo (e) Pyrene	1.1	1.1
Benzo (a) Pyrene	0.44	0.44
Perylene	0.22	0.22
Indeno (1,2,3 - cd) Pyrene	0.11	0.11
Dibenzo (a,h) anthracene	0.07	0.07
Benzo (g,h,i) perylene	0.16	0.16
Total Aromatics	4200	4200
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00
Phenanthrene d-10	1.00	1.00

TREATMENT REPLICATE 1
Testing Date: Day 7
Initial Oil Weight: 500 mg
Final Extracted Volume: 10.0 mL

	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	6.5	6.5
nC-11 Undecane	10	10
nC-12 Dodecane	6.2	6.2
nC-13 Tridecane	2.2	2.2
nC-14 Tetradecane	33	33
nC-15 Pentadecane	270	270
nC-16 Hexadecane	370	370
nC-17 Heptadecane	450	450
Pristane	990	990
nC-18 Octadecane	450	450
Phytane	690	690
nC-19 Nonadecane	560	560
nC-20 Eicosane	610	610
nC-21 Heneicosane	550	550
nC-22 Docosane	550	550
nC-23 Tricosane	570	570
nC-24 Tetracosane	570	570
nC-25 Pentacosane	570	570
nC-26 Hexacosane	560	560
nC-27 Heptacosane	460	460
nC-28 Octacosane	450	450
nC-29 Nonacosane	360	360
nC-30 Triacontane	390	390
nC-31 Hentriacontane	400	400
nC-32 Dotriacontane	350	350
nC-33 Tritriacontane	370	370
nC-34 Tetratriacontane	250	250
nC-35 Pentatriacontane	270	270
Total Alkanes	11000	11000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	0.84	0.84
C1-Naphthalenes	4.3	4.3
C2-Naphthalenes	170	170
C3-Naphthalenes	540	540
C4-Naphthalenes	360	360
Fluorene	45	45
C1-Fluorenes	150	150
C2-Fluorenes	160	160
C3- Fluorenes	160	160
Dibenzothiophene	140	140
C1-Dibenzothiophenes	210	210
C2-Dibenzothiophenes	250	250
C3- Dibenzothiophenes	160	160
Phenanthrene	170	170
C1-Phenanthrenes	330	330
C2-Phenanthrenes	330	330
C3-Phenanthrenes	190	190
C4-Phenanthrenes	96	96
Anthracene	0.18	0.18
Fluoranthene	3.5	3.5
Pyrene	1.7	1.7
C1- Pyrenes	55	55
C2- Pyrenes	73	73
C3- Pyrenes	74	74
C4- Pyrenes	53	53
Naphthobenzothiophene	34	34
C-1 Naphthobenzothiophenes	130	130
C-2 Naphthobenzothiophenes	130	130
C-3 Naphthobenzothiophenes	100	100
Benzo (a) Anthracene	2.5	2.5
Chrysene	41	41
C1- Chrysenes	65	65
C2- Chrysenes	82	82
C3- Chrysenes	69	69
C4- Chrysenes	55	55
Benzo (b) Fluoranthene	1.0	1.0
Benzo (k) Fluoranthene	1.1	1.1
Benzo (e) Pyrene	1.3	1.3
Benzo (a) Pyrene	0.41	0.41
Perylene	0.22	0.22
Indeno (1,2,3 - cd) Pyrene	0.11	0.11
Dibenzo (a,h) anthracene	0.07	0.07
Benzo (g,h,i) perylene	0.12	0.12
Total Aromatics	4400	4400
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00
Phenanthrene d-10	1.00	1.00

TREATMENT REPLICATE 2
Testing Date: Day 7
Initial Oil Weight: 510 mg
Final Extracted Volume: 10.1 mL

	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	6.2	6.2
nC-11 Undecane	11	11
nC-12 Dodecane	6.1	6.1
nC-13 Tridecane	2.3	2.3
nC-14 Tetradecane	32	32
nC-15 Pentadecane	280	280
nC-16 Hexadecane	380	380
nC-17 Heptadecane	430	430
Pristane	930	930
nC-18 Octadecane	430	430
Phytane	730	730
nC-19 Nonadecane	530	530
nC-20 Eicosane	570	570
nC-21 Heneicosane	510	510
nC-22 Docosane	510	510
nC-23 Tricosane	520	520
nC-24 Tetracosane	560	560
nC-25 Pentacosane	540	540
nC-26 Hexacosane	520	520
nC-27 Heptacosane	410	410
nC-28 Octacosane	400	400
nC-29 Nonacosane	330	330
nC-30 Triacontane	350	350
nC-31 Hentriacontane	400	400
nC-32 Dotriacontane	340	340
nC-33 Tritriacontane	360	360
nC-34 Tetratriacontane	250	250
nC-35 Pentatriacontane	280	280
Total Alkanes	11000	11000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	0.88	0.88
C1-Naphthalenes	4.4	4.4
C2-Naphthalenes	180	180
C3-Naphthalenes	560	560
C4-Naphthalenes	370	370
Fluorene	42	42
C1-Fluorenes	140	140
C2-Fluorenes	150	150
C3- Fluorenes	150	150
Dibenzothiophene	140	140
C1-Dibenzothiophenes	200	200
C2-Dibenzothiophenes	230	230
C3- Dibenzothiophenes	160	160
Phenanthrene	160	160
C1-Phenanthrenes	310	310
C2-Phenanthrenes	320	320
C3-Phenanthrenes	180	180
C4-Phenanthrenes	91	91
Anthracene	0.18	0.18
Fluoranthene	3.4	3.4
Pyrene	1.7	1.7
C1- Pyrenes	60	60
C2- Pyrenes	73	73
C3- Pyrenes	74	74
C4- Pyrenes	56	56
Naphthobenzothiophene	34	34
C-1 Naphthobenzothiophenes	130	130
C-2 Naphthobenzothiophenes	140	140
C-3 Naphthobenzothiophenes	100	100
Benzo (a) Anthracene	2.7	2.7
Chrysene	41	41
C1- Chrysenes	67	67
C2- Chrysenes	80	80
C3- Chrysenes	70	70
C4- Chrysenes	56	56
Benzo (b) Fluoranthene	1.0	1.0
Benzo (k) Fluoranthene	1.1	1.1
Benzo (e) Pyrene	1.4	1.4
Benzo (a) Pyrene	0.54	0.54
Perylene	0.25	0.25
Indeno (1,2,3 - cd) Pyrene	0.11	0.11
Dibenzo (a,h) anthracene	0.06	0.06
Benzo (g,h,i) perylene	0.10	0.10
Total Aromatics	4400	4400
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00
Phenanthrene d-10	1.00	1.00

TREATMENT REPLICATE 3
Testing Date: Day 7
Initial Oil Weight: 510 mg
Final Extracted Volume: 10.0 mL

	Concentration (ng/mg)	Surrogate Corrected (ng/mg)
Alkane Analyte:		
nC-10 Decane	6.6	6.6
nC-11 Undecane	12	12
nC-12 Dodecane	6.3	6.3
nC-13 Tridecane	2.2	2.2
nC-14 Tetradecane	34	34
nC-15 Pentadecane	320	320
nC-16 Hexadecane	400	400
nC-17 Heptadecane	440	440
Pristane	930	930
nC-18 Octadecane	480	480
Phytane	740	740
nC-19 Nonadecane	580	580
nC-20 Eicosane	560	560
nC-21 Heneicosane	580	580
nC-22 Docosane	580	580
nC-23 Tricosane	650	650
nC-24 Tetracosane	550	550
nC-25 Pentacosane	560	560
nC-26 Hexacosane	550	550
nC-27 Heptacosane	460	460
nC-28 Octacosane	460	460
nC-29 Nonacosane	390	390
nC-30 Triacontane	410	410
nC-31 Hentriacontane	440	440
nC-32 Dotriacontane	350	350
nC-33 Tritriacontane	330	330
nC-34 Tetratriacontane	210	210
nC-35 Pentatriacontane	220	220
Total Alkanes	11000	11000
5 Alpha Androstane	2000	2000
Aromatic Analyte:		
Naphthalene	0.84	0.84
C1-Naphthalenes	4.5	4.5
C2-Naphthalenes	180	180
C3-Naphthalenes	560	560
C4-Naphthalenes	370	370
Fluorene	40	40
C1-Fluorenes	140	140
C2-Fluorenes	150	150
C3- Fluorenes	150	150
Dibenzothiophene	140	140
C1-Dibenzothiophenes	210	210
C2-Dibenzothiophenes	240	240
C3- Dibenzothiophenes	170	170
Phenanthrene	160	160
C1-Phenanthrenes	320	320
C2-Phenanthrenes	330	330
C3-Phenanthrenes	200	200
C4-Phenanthrenes	92	92
Anthracene	0.20	0.20
Fluoranthene	3.3	3.3
Pyrene	1.6	1.6
C1- Pyrenes	49	49
C2- Pyrenes	71	71
C3- Pyrenes	71	71
C4- Pyrenes	57	57
Naphthobenzothiophene	32	32
C-1 Naphthobenzothiophenes	130	130
C-2 Naphthobenzothiophenes	150	150
C-3 Naphthobenzothiophenes	97	97
Benzo (a) Anthracene	3.0	3.0
Chrysene	42	42
C1- Chrysenes	69	69
C2- Chrysenes	80	80
C3- Chrysenes	66	66
C4- Chrysenes	52	52
Benzo (b) Fluoranthene	0.86	0.86
Benzo (k) Fluoranthene	0.94	0.94
Benzo (e) Pyrene	1.1	1.1
Benzo (a) Pyrene	0.35	0.35
Perylene	0.20	0.20
Indeno (1,2,3 - cd) Pyrene	0.09	0.09
Dibenzo (a,h) anthracene	0.07	0.07
Benzo (g,h,i) perylene	0.12	0.12
Total Aromatics	4400	4400
Phenanthrene d-10	2000	2000
% Surrogate Recovery		
5 Alpha Androstane	1.00	1.00
Phenanthrene d-10	1.00	1.00

Sample Decription	Treatment	Treatment	Treatment	Control	Control	Control
Time and Replicate	Day 28, Rep# 1	Day 28, Rep# 2	Day 28, Rep# 3	Day 28, Rep# 1	Day 28, Rep# 2	Day 28, Rep# 3
Final Volume (mL)	10	10	10	10	10	10
Mass Extracted (mg)	416	408	400	408	400	400
Surrogate#1 Recovery (%)	95%	99%	87%	101%	105%	96%
Surrogate#2 Recovery (%)	108%	109%	102%	105%	109%	102%
Compounds:	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)
Naphthalene-d8 IS #1	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-10 Decane	0.000	0.000	0.000	0.000	0.000	0.000
nC-11 Undecane	0.000	0.000	0.000	0.283	0.179	0.832
nC-12 Dodecane	0.000	0.000	0.000	0.839	0.735	1.022
nC-13 Tridecane	0.000	0.000	0.000	2.42	2.19	1.96
nC-14 Tetradecane	0.127	0.101	0.116	93.1	82.0	87.7
Naphthalene	0.174	0.142	0.148	0.114	0.164	0.178
C1-Naphthalenes	2.88	2.34	2.75	2.160	2.150	2.120
C2-Naphthalenes	189	175	174	264.0	253.0	223.0
C3-Naphthalenes	717	646	712	773	753	795
C4-Naphthalenes	564	494	512	521	573	570
Acenaphthene-d10 IS #2	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-15 Pentadecane	0.000	3.382	0.000	802	779	736
nC-16 Hexadecane	3.71	2.99	3.48	1639	1553	1465
nC-17 Heptadecane	15.7	17.2	12.9	2067	1863	1637
Pristane	342	197	77.4	1393	1357	1184
nC-18 Octadecane	6.29	7.98	8.24	1845	1740	1542
Phytane	420	157	60.8	898	901	801
nC-19 Nonadecane	54.3	44.9	31.6	1823	1743	1475
nC-20 Eicosane	68.1	36.3	36.4	1734	1623	1509
nC-21 Heneicosane	22.4	13.4	10.1	1357	1295	1150
nC-22 Docosane	6.86	8.51	9.84	1207	1134	1004
nC-23 Tricosane	3.89	3.38	3.41	1047	985	903
Fluorene	48.7	45.5	51.3	69.2	65.2	69.2
C1-Fluorenes	189	181	204	210	219	216
C2-Fluorenes	203	184	187	211	203	208
C3- Fluorenes	208	202	205	234	215	203
Dibenzothiophene	165	158	167	169	190.0	188.0
C1-Dibenzothiophenes	257	240	246	241	221	242
C2-Dibenzothiophenes	270	272	272	296	282	275
C3- Dibenzothiophenes	182	185	181	212	197	208
Phenanthrene	236	227	236	223	294	266
C1-Phenanthrenes	398	380	400	402	455	413
C2-Phenanthrenes	402	410	411	454	428	481
C3-Phenanthrenes	233	255	273	310	287	263
C4-Phenanthrenes	92.1	96.0	79.0	110	101	92.1
Anthracene	0.798	0.470	0.574	0.643	0.685	0.668
Phenanthrene-d10 SS #1	1000	1000	1000	1000	1000	1000
5-alpha Androstane SS #2	1000	1000	1000	1000	1000	1000
Chrysene-d12 IS #3	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-24 Tetracosane	12.5	10.1	10.2	601	596	598
nC-25 Pentacosane	7.02	7.14	6.57	534	538	538
nC-26 Hexacosane	4.67	4.29	4.65	518	502	498
nC-27 Heptacosane	2.16	1.91	1.81	409	394	388
nC-28 Octacosane	2.24	1.98	2.26	364	351	359
Fluoranthene	4.30	3.69	3.87	3.49	3.36	3.13
Pyrene	4.31	3.53	3.75	3.33	3.49	3.51
C1- Pyrenes	43.8	38.3	41.2	43.6	50.1	39.0
C2- Pyrenes	65.3	60.4	60.1	66.4	63.2	62.1
C3- Pyrenes	70.7	70.4	66.0	72.6	75.4	74.4
C4- Pyrenes	41.8	40.1	40.3	50.4	45.6	46.3
Naphthobenzothiophene	26.8	24.5	26.6	28.2	26.3	26.3
C-1 Naphthobenzothiophenes	83.3	78.4	78.9	95.3	95.7	93.1
C-2 Naphthobenzothiophenes	101	98	103	117	111	106
C-3 Naphthobenzothiophenes	78.6	78.2	76.9	94.1	83.9	81.4
Benzo (a) Anthracene	6.31	4.49	6.37	6.90	4.75	5.39
Chrysene	40.6	37.8	40.5	40.8	42.1	38.7
C1- Chrysenes	67.5	60.4	63.7	67.4	64.0	65.5
C2- Chrysenes	72.5	72.5	67.4	89.2	83.7	78.3
C3- Chrysenes	66.1	71.3	66.7	78.6	74.9	73.4
C4- Chrysenes	39.6	38.6	36.7	47.0	42.9	41.4
Perylene-d12 IS #4	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-29 Nonacosane	2.172	4.283	3.897	221	219	214
nC-30 Triacontane	1.298	5.582	6.488	219	215	222
nC-31 Hentriacontane	3.186	7.625	7.634	238	228	228
nC-32 Dotriacontane	2.047	9.188	9.598	221	214	201
nC-33 Tritriacontane	1.502	10.888	8.914	206	204	200
nC-34 Tetratriacontane	0.514	22.484	31.349	222	174	180
nC-35 Pentatriacontane	2.391	33.259	36.020	323	286	225
Benzo (b) Fluoranthene	0.787	0.544	0.706	1.43	1.23	1.74
Benzo (k) Fluoranthene	2.088	2.510	2.380	2.17	2.12	2.65
Benzo (e) Pyrene	0.406	0.516	0.433	0.899	0.680	0.772
Benzo (a) Pyrene	1.000	1.084	1.111	0.979	1.51	1.13
Perylene	0.228	0.231	0.847	0.654	0.857	0.516
Indeno (1,2,3 - cd) Pyrene	0.000	0.000	0.000	0.000	0.050	0.006
Dibenzo (a,h) anthracene	0.000	0.000	0.000	0.000	0.013	0.019
Benzo (g,h,i) perylene	0.413	0.053	0.469	1.57	0.930	1.01
Total Alkanes	985	611	384	19985	18979	17348
Total Aromatics	5177	4941	5101	5614	5616	5561

Sample Decription	Treatment	Treatment	Treatment	Control	Control	Control
Time and Replicate	Day 28, Rep# 1	Day 28, Rep# 2	Day 28, Rep# 3	Day 28, Rep# 1	Day 28, Rep# 2	Day 28, Rep# 3
Final Volume (mL)	10	10	10	10	10	10
Mass Extracted (mg)	416	408	400	408	400	400
Surrogate#1 Recovery (%)	95%	99%	87%	101%	105%	96%
Surrogate#2 Recovery (%)	108%	109%	102%	105%	109%	102%
Compounds:	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)	Conc (ng/mg)
Naphthalene-d8 IS #1	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-10 Decane	0.000	0.000	0.000	0.000	0.000	0.000
nC-11 Undecane	0.000	0.000	0.000	0.283	0.179	0.832
nC-12 Dodecane	0.000	0.000	0.000	0.839	0.735	1.022
nC-13 Tridecane	0.000	0.000	0.000	2.42	2.19	1.96
nC-14 Tetradecane	0.127	0.101	0.116	93.1	82.0	87.7
Naphthalene	0.174	0.142	0.148	0.114	0.164	0.178
C1-Naphthalenes	2.88	2.34	2.75	2.160	2.150	2.120
C2-Naphthalenes	189	175	174	264.0	253.0	223.0
C3-Naphthalenes	717	646	712	773	753	795
C4-Naphthalenes	564	494	512	521	573	570
Acenaphthene-d10 IS #2	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-15 Pentadecane	0.000	3.382	0.000	802	779	736
nC-16 Hexadecane	3.71	2.99	3.48	1639	1553	1465
nC-17 Heptadecane	15.7	17.2	12.9	2067	1863	1637
Pristane	342	197	77.4	1393	1357	1184
nC-18 Octadecane	6.29	7.98	8.24	1845	1740	1542
Phytane	420	157	60.8	898	901	801
nC-19 Nonadecane	54.3	44.9	31.6	1823	1743	1475
nC-20 Eicosane	68.1	36.3	36.4	1734	1623	1509
nC-21 Henelcosane	22.4	13.4	10.1	1357	1295	1150
nC-22 Docosane	6.86	8.51	9.84	1207	1134	1004
nC-23 Tricosane	3.89	3.38	3.41	1047	985	903
Fluorene	48.7	45.5	51.3	69.2	65.2	69.2
C1-Fluorenes	189	181	204	210	219	216
C2-Fluorenes	203	184	187	211	203	208
C3- Fluorenes	208	202	205	234	215	203
Dibenzothiophene	165	158	167	169	190.0	188.0
C1-Dibenzothiophenes	257	240	246	241	221	242
C2-Dibenzothiophenes	270	272	272	296	282	275
C3- Dibenzothiophenes	182	185	181	212	197	208
Phenanthrene	236	227	236	223	294	266
C1-Phenanthrenes	398	380	400	402	455	413
C2-Phenanthrenes	402	410	411	454	428	481
C3-Phenanthrenes	233	255	273	310	287	263
C4-Phenanthrenes	92.1	96.0	79.0	110	101	92.1
Anthracene	0.798	0.470	0.574	0.643	0.685	0.668
Phenanthrene-d10 SS #1	1000	1000	1000	1000	1000	1000
5-alpha Androstane SS #2	1000	1000	1000	1000	1000	1000
Chrysene-d12 IS #3	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-24 Tetracosane	12.5	10.1	10.2	601	596	598
nC-25 Pentacosane	7.02	7.14	6.57	534	538	538
nC-26 Hexacosane	4.67	4.29	4.65	518	502	498
nC-27 Heptacosane	2.16	1.91	1.81	409	394	388
nC-28 Octacosane	2.24	1.98	2.26	364	351	359
Fluoranthene	4.30	3.69	3.87	3.49	3.36	3.13
Pyrene	4.31	3.53	3.75	3.33	3.49	3.51
C1- Pyrenes	43.8	38.3	41.2	43.6	50.1	39.0
C2- Pyrenes	65.3	60.4	60.1	66.4	63.2	62.1
C3- Pyrenes	70.7	70.4	66.0	72.6	75.4	74.4
C4- Pyrenes	41.8	40.1	40.3	50.4	45.6	46.3
Naphthobenzothiophene	26.8	24.5	26.6	28.2	26.3	26.3
C-1 Naphthobenzothiophenes	83.3	78.4	78.9	95.3	95.7	93.1
C-2 Naphthobenzothiophenes	101	98	103	117	111	106
C-3 Naphthobenzothiophenes	78.6	78.2	76.9	94.1	83.9	81.4
Benzo (a) Anthracene	6.31	4.49	6.37	6.90	4.75	5.39
Chrysene	40.6	37.8	40.5	40.8	42.1	38.7
C1- Chrysenes	67.5	60.4	63.7	67.4	64.0	65.5
C2- Chrysenes	72.5	72.5	67.4	89.2	83.7	78.3
C3- Chrysenes	66.1	71.3	66.7	78.6	74.9	73.4
C4- Chrysenes	39.6	38.6	36.7	47.0	42.9	41.4
Perylene-d12 IS #4	IntStd	IntStd	IntStd	IntStd	IntStd	IntStd
nC-29 Nonacosane	2.172	4.283	3.897	221	219	214
nC-30 Triacontane	1.298	5.582	6.488	219	215	222
nC-31 Hentriacontane	3.186	7.625	7.634	238	228	228
nC-32 Dotriacontane	2.047	9.188	9.598	221	214	201
nC-33 Tritriacontane	1.502	10.888	8.914	206	204	200
nC-34 Tetratriacontane	0.514	22.484	31.349	222	174	180
nC-35 Pentatriacontane	2.391	33.259	36.020	323	286	225
Benzo (b) Fluoranthene	0.787	0.544	0.706	1.43	1.23	1.74
Benzo (k) Fluoranthene	2.088	2.510	2.380	2.17	2.12	2.65
Benzo (e) Pyrene	0.406	0.516	0.433	0.899	0.680	0.772
Benzo (a) Pyrene	1.000	1.084	1.111	0.979	1.51	1.13
Perylene	0.228	0.231	0.847	0.654	0.857	0.516
Indeno (1,2,3 - cd) Pyrene	0.000	0.000	0.000	0.000	0.050	0.006
Dibenzo (a,h) anthracene	0.000	0.000	0.000	0.000	0.013	0.019
Benzo (g,h,i) perylene	0.413	0.053	0.469	1.57	0.930	1.01
Total Alkanes	985	611	384	19985	18979	17348
Total Aromatics	5177	4941	5101	5614	5616	5561

BIO-AQUATIC TESTING, INC.
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

Lab ID NA (For Use By Bio-Aquatic Testing Only)

For 48-Hr or Chronic Test Schedules, Please mark which sample this is: ____First, ____Second, or ____Third.

Client: IEP Corp (Brazos & Dayton Project)
Facility: NA - Sampling Location
Permit No: NA
~~Outfall~~: SEAWATER SAMPLE

Sample Type: ○ Composite ○ Grab
Dilution Water: ○ ~~Receiving Stream~~ ○ ~~Synthetic Lab~~ NA
Dechlorinate Sample? ○ Yes ○ No

If Necessary, Make Changes or Additions to Test Information Here:

TEST(S) NEEDED	Freshwater	Saltwater
☐ Chronic	☐ Pimephales promelas-Fathead	☐ Cyprinodon variegatus-Sheepshead
☐ 96 Hour Acute	☐ Ceriodaphnia dubia	☐ Menidia beryllina-Silverside
☐ 48 Hour Acute	☐ Daphnia pulex	☐ Mysidopsis bahia
☐ 24 Hour Acute	☐ Daphnia magna	

NA

Dilution Series: _____

BELOW IS THE TEST WE HAVE SCHEDULED. PLEASE MAKE SURE THE TEST INFORMATION IS CORRECT. YOU CAN MAKE ANY CHANGES OR ADDITIONS IN THE BOX TO THE LEFT.

TEST INFORMATION

Type Of Test NA Test Organism

Dilution Series: _____

Do you need 24-hr acute (100%) data ___Yes ___No

Outfall Number/Nam	Person Performing Sampling	Start Date	Start Time	End Date	End Time	Number Of Portions Composited	Methods Of Collections And Composite: Auto Coll. Auto Comp.	Manual Coll. Manual Comp.	Auto Coll. Manual Comp.	Number Of Containers Shipped
Galveston Bay	Bennett	2/11	2:00 PM			4		✓		2

Receiving Stream Name and	Person Performing Sampling	Sample Collected Date	Sample Collected Time	Number Of Containers Shipped

Method of Shipment: ☑ Greyhound ☐ Pony Express ☐ Federal Express ☐ UPS Next Day ☐ Client Delivered ☐ Bio-Pick Up ☐ Delta Dash ☐ American Airlines ☐ Southwest Airlines ☐ Other _____

Relinquished By: _____ Date 2/11/02 Time 5:00 PM Received By: _____ Date 2/12/02 Time 8:00 AM

Relinquished By: _____ Date _____ Time _____ Received By: _____ Date _____ Time _____

Received For Bio-Aquatic By: _____ Date _____ Time _____ Temperature (C) _____ Condition _____

Samples received after 4:30pm will be logged in the following day.

Chlorine _____ mg/l Salinity _____ ppt

Bio-Aquatic Testing

Chain of Custody Record

Company Bio-Aquatic Testing, Inc.
Address 2601 Mayes Rd. Suite 100
Phone 972-242-7750 Fax 7749
Project Name/ Location Joe Doughtery Bioremediation
Project Number NA
Project Manager Marc Bentley

Report To: Marc Bentley
Invoice To: Bio-Aquatic Testing, Inks
P.O. No. _____

SAMPLED BY
Marc A. Bentley (PRINT NAME) Marc A. Bentley SIGNATURE
_____ (PRINT NAME) _____ SIGNATURE

DATE	TIME	SAMPLE DESCRIPTION	GC/MS	COMMENTS
4/29/02	5:15	Frozen water & oil samples	✓	
		Day 0 Reps # 1-3 Control	✓	
		Day 0 Reps # 1-3 Treatment	✓	
		Day 7 Reps # 1-3 Control	✓	
		Day 7 Reps # 1-3 Treatment	✓	
		TOTAL of 12 samples		

CONDITION OF SAMPLE: good
TEMPERATURE: 4° Celcius Bottles Supplied by Bio-Aquatic Testing? Yes ✓ No ___
SAMPLE REMAINDER: Return sample remainder via UPS
I request Bio to dispose of all sample remainder N/A

Relinquished By:	Date/Time	Received By:	Relinquished By:	Date/Time	Received By:
Marc A. Bentley	4/29/02				

Received for Bio-Aquatic By:	Date/Time	Method of Shipment	Remarks
		UPS Overnight	Please return ice chest & empty flasks via ground UPS.

Bio-Aquatic Testing

Chain of Custody Record

Company: Bio-Aquatic Testing, Inc
Address: 2501 Mayes Rd. Suite 100
Phone: (972) 242-7750 ext 41 Fax: (972) 242-7749
Project Name/ Location: Jim Lynn / PA.
Project Number: N/A
Project Manager: Marc Bentley

Report To: Marc Bentley
Invoice To: Bio-Aquatic Testing Inc.
P.O. No.:

SAMPLED BY
Marc Bentley (PRINT NAME) — Marc D. Bentley (SIGNATURE)
(PRINT NAME) — (SIGNATURE)

ANALYSES: GC/MS ✓

DATE	TIME	SAMPLE DESCRIPTION	COMMENTS
4/1/02	5:30 pm	6-250 ml oil/water mixture flasks	Samples from Day 28 sacrifice
4/1/02	5:30 pm	6-20 ml vials for sampling extract. To be returned ~5 ml of extract from final volume of 20 ml.	

CONDITION OF SAMPLE: Good
TEMPERATURE: 4° Celsius — Bottles Supplied by Bio-Aquatic Testing? Yes ✓ No
SAMPLE REMAINDER: Return sample remainder via Ground Delivery
I request Bio to dispose of all sample remainder N/A

Relinquished By:	Date/Time	Received By:	Relinquished By:	Date/Time	Received By:
Marc D. Bentley	5:30pm/4/1/02	Buffy M. Miller			

Received for Bio-Aquatic By:	Date/Time	Method of Shipment	Remarks
		UPS overnight	

GC/MS DATA

STATISTICAL ANALYSIS

COMPUTER PRINTOUTS

APPENDIX II

General Linear Model: Alkanes versus Treatments, Day

```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Alkanes, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen         1  358512790  358512790  358512790  442.33  0.000
Day              2 1093189029 1093189029  546594515  674.38  0.000
Treatmen*Day     2  297814914  297814914  148907457  183.72  0.000
Error           12    9726191    9726191     810516
Total           17 1759242924

Unusual Observations for Alkanes

Obs    Alkanes       Fit      SE Fit  Residual   St Resid
  3    26000.0   27666.7       519.8   -1666.7     -2.27R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Alkanes
Comparisons with Control Level
Treatmen = Control
Day      = 0 subtracted from:

               Difference
Treatmen*Day   of Means       Upper  -+---------+---------+---------+-----
Control    7      -6333       -4494  ---------------------------*)
Control   28      -8896       -7057  -------------------------*)
Product    0       1667        3506  ---------------------------------*-)
Product    7     -16667      -14827  -----------------*-)
Product   28     -27007      -25167  --------*-)
                                     -+---------+---------+---------+-----
                                   -36000    -24000    -12000          0


Dunnett Simultaneous Tests
Response Variable Alkanes
Comparisons with Control Level
Treatmen = Control
Day      = 0 subtracted from:

Level             Difference       SE of              Adjusted
Treatmen*Day       of Means  Difference   T-Value    P-Value
Control    7          -6333       735.1     -8.62     0.0000
Control   28          -8896       735.1    -12.10     0.0000
Product    0           1667       735.1      2.27     0.9994
Product    7         -16667       735.1    -22.67     0.0000
Product   28         -27007       735.1    -36.74     0.0000
```

General Linear Model: Alkanes versus Treatments, Day

```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Alkanes, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatmen	1	358512790	358512790	358512790	442.33	0.000
Day	2	1093189029	1093189029	546594515	674.38	0.000
Treatmen*Day	2	297814914	297814914	148907457	183.72	0.000
Error	12	9726191	9726191	810516		
Total	17	1759242924				

Unusual Observations for Alkanes

Obs	Alkanes	Fit	SE Fit	Residual	St Resid
3	26000.0	27666.7	519.8	-1666.7	-2.27R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Alkanes
Comparisons with Control Level
Treatmen = Control
Day = 7 subtracted from:

Treatmen*Day		Difference of Means	Upper	-----+---------+---------+---------+-
Control	0	6333	8173	----------*-)
Control	28	-2563	-723	----------*)
Product	0	8000	9839	----------*)
Product	7	-10333	-8494	*-)
Product	28	-20673	-18834	*)
				-----+---------+---------+---------+-
				-24000 -12000 0 12000

Dunnett Simultaneous Tests
Response Variable Alkanes
Comparisons with Control Level
Treatmen = Control
Day = 7 subtracted from:

Level Treatmen*Day		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Control	0	6333	735.1	8.62	1.0000
Control	28	-2563	735.1	-3.49	0.0089
Product	0	8000	735.1	10.88	1.0000
Product	7	-10333	735.1	-14.06	0.0000
Product	28	-20673	735.1	-28.12	0.0000

General Linear Model: Alkanes versus Treatments, Day

Factor	Type	Levels	Values
Treatmen	fixed	2	Control Product
Day	fixed	3	0 7 28

Analysis of Variance for Alkanes, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatmen	1	358512790	358512790	358512790	442.33	0.000
Day	2	1093189029	1093189029	546594515	674.38	0.000
Treatmen*Day	2	297814914	297814914	148907457	183.72	0.000
Error	12	9726191	9726191	810516		
Total	17	1759242924				

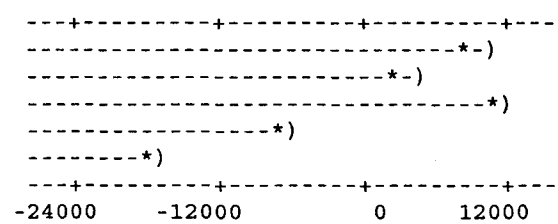
Unusual Observations for Alkanes

Obs	Alkanes	Fit	SE Fit	Residual	St Resid
3	26000.0	27666.7	519.8	-1666.7	-2.27R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Alkanes
Comparisons with Control Level
Treatmen = Control
Day = 28 subtracted from:

Treatmen*Day		Difference of Means	Upper
Control	0	8896	10735
Control	7	2563	4402
Product	0	10563	12402
Product	7	-7771	-5931
Product	28	-18111	-16271



Dunnett Simultaneous Tests
Response Variable Alkanes
Comparisons with Control Level
Treatmen = Control
Day = 28 subtracted from:

Level Treatmen*Day		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Control	0	8896	735.1	12.10	1.0000
Control	7	2563	735.1	3.49	1.0000
Product	0	10563	735.1	14.37	1.0000
Product	7	-7771	735.1	-10.57	0.0000
Product	28	-18111	735.1	-24.64	0.0000

} These are significantly less than Day 28 Control

General Linear Model: Aromatics versus Treatments, Day

```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Aromatic, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen         1      76310      76310      76310   10.86  0.006
Day              2    5262233    5262233    2631117  374.52  0.000
Treatmen*Day     2     348887     348887     174444   24.83  0.000
Error           12      84303      84303       7025
Total           17    5771734

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Aromatic
Comparisons with Control Level
Treatmen = Control
Day      = 0 subtracted from:

              Difference
Treatmen*Day  of Means       Upper  ---------+---------+---------+-------
Control   7      -1267        -1095  --------*--)
Control  28         -3          168  -------------------------------*--)
Product   0         67          238  -------------------------------*--)
Product   7      -1200        -1029  ---------*--)
Product  28       -527         -356  ---------------------*--)
                                     ---------+---------+---------+-------
                                           -1200      -600         0

Dunnett Simultaneous Tests
Response Variable Aromatic
Comparisons with Control Level
Treatmen = Control
Day      = 0 subtracted from:

Level             Difference       SE of              Adjusted
Treatmen*Day       of Means  Difference   T-Value    P-Value
Control   7           -1267       68.44    -18.51     0.0000
Control  28              -3       68.44     -0.04     0.8202
Product   0              67       68.44      0.97     0.9803
Product   7           -1200       68.44    -17.53     0.0000
Product  28            -527       68.44     -7.70     0.0000
```

General Linear Model: Aromatics versus Treatments, Day

```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Aromatic, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen         1      76310      76310      76310   10.86  0.006
Day              2    5262233    5262233    2631117  374.52  0.000
Treatmen*Day     2     348887     348887     174444   24.83  0.000
Error           12      84303      84303       7025
```

```
Total          17    5771734

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Aromatic
Comparisons with Control Level
Treatmen = Control
Day      = 7 subtracted from:

                Difference
Treatmen*Day    of Means       Upper  -------+---------+---------+---------
Control    0    1266.67       1437.9  ----------------------------*--)
Control   28    1263.67       1434.9  ----------------------------*--)
Product    0    1333.33       1504.6  -----------------------------*--)
Product    7      66.67        237.9  --------*--)
Product   28     739.67        910.9  -------------------*--)
                                      -------+---------+---------+---------
                                             0       600      1200


Dunnett Simultaneous Tests
Response Variable Aromatic
Comparisons with Control Level
Treatmen = Control
Day      = 7 subtracted from:

Level              Difference       SE of              Adjusted
Treatmen*Day         of Means  Difference   T-Value     P-Value
Control    0         1266.67       68.44   18.5087      1.0000
Control   28         1263.67       68.44   18.4649      1.0000
Product    0         1333.33       68.44   19.4829      1.0000
Product    7           66.67       68.44    0.9741      0.9803
Product   28          739.67       68.44   10.8081      1.0000
```

General Linear Model: Aromatics versus Treatments, Day

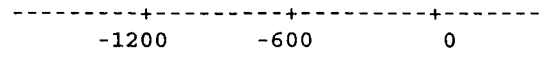
```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Aromatic, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen         1      76310      76310      76310   10.86  0.006
Day              2    5262233    5262233    2631117  374.52  0.000
Treatmen*Day     2     348887     348887     174444   24.83  0.000
Error           12      84303      84303       7025
Total           17    5771734

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Aromatic
Comparisons with Control Level
Treatmen = Control
Day      = 28 subtracted from:

                Difference
Treatmen*Day    of Means       Upper  ---------+---------+---------+-------
Control    0           3         174  -----------------------------*--)
Control    7       -1264       -1092  --------*--)
Product    0          70         241  -------------------------------*--)
Product    7       -1197       -1026  ---------*--)
Product   28        -524        -353  --------------------*--)
```



Dunnett Simultaneous Tests
Response Variable Aromatic
Comparisons with Control Level
Treatmen = Control
Day = 28 subtracted from:

Level Treatmen	*Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Control	0	3	68.44	0.04	0.8458
Control	7	-1264	68.44	-18.46	0.0000
Product	0	70	68.44	1.02	0.9824
Product	7	-1197	68.44	-17.49	0.0000
Product	28	-524	68.44	-7.66	0.0000

} These are significantly less than Day 28 Controls

General Linear Model: Ranked aromatics versus Treatments, Day

```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Ranked a, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen         1      2.000      2.000      2.000    0.43  0.523
Day              2    364.333    364.333    182.167   39.51  0.000
Treatmen*Day     2     50.333     50.333     25.167    5.46  0.021
Error           12     55.333     55.333      4.611
Total           17    472.000

Unusual Observations for Ranked a

Obs  Ranked a       Fit      SE Fit  Residual   St Resid
  2   17.0000   13.1667      1.2398    3.8333      2.19R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Ranked a
Comparisons with Control Level
Treatmen = Control
Day      = 0 subtracted from:

                Difference
Treatmen*Day    of Means       Upper  ------+---------+---------+---------+
Control   7      -10.17      -5.779  ---------*------)
Control  28        0.17       4.554  ----------------------------*-------)
Product   0        2.33       6.721  --------------------------------*------)
Product   7       -9.17      -4.779  -----------*------)
Product  28       -5.17      -0.779  -----------------*-------)
                                     ------+---------+---------+---------+
                                        -12.0      -6.0       0.0       6.0


Dunnett Simultaneous Tests
Response Variable Ranked a
Comparisons with Control Level
Treatmen = Control
Day      = 0 subtracted from:

Level             Difference       SE of              Adjusted
Treatmen*Day       of Means  Difference   T-Value    P-Value
Control   7         -10.17       1.753    -5.799     0.0002
Control  28           0.17       1.753     0.095     0.8596
Product   0           2.33       1.753     1.331     0.9922
Product   7          -9.17       1.753    -5.228     0.0005
Product  28          -5.17       1.753    -2.947     0.0231
```

General Linear Model: Ranked aromatics versus Treatments, Day

```
Factor     Type Levels Values
Treatmen   fixed      2 Control Product
Day        fixed      3  0  7 28

Analysis of Variance for Ranked a, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatmen	1	2.000	2.000	2.000	0.43	0.523
Day	2	364.333	364.333	182.167	39.51	0.000
Treatmen*Day	2	50.333	50.333	25.167	5.46	0.021
Error	12	55.333	55.333	4.611		
Total	17	472.000				

Unusual Observations for Ranked a

Obs	Ranked a	Fit	SE Fit	Residual	St Resid
2	17.0000	13.1667	1.2398	3.8333	2.19R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Ranked a
Comparisons with Control Level
Treatmen = Control
Day = 7 subtracted from:

Treatmen*Day		Difference of Means	Upper	-------+---------+---------+---------
Control	0	10.167	14.554	------------------------*------)
Control	28	10.333	14.721	------------------------*-------)
Product	0	12.500	16.887	----------------------------*------)
Product	7	1.000	5.387	---------*------)
Product	28	5.000	9.387	----------------*-------)
				-------+---------+---------+---------
				0.0 6.0 12.0

Dunnett Simultaneous Tests
Response Variable Ranked a
Comparisons with Control Level
Treatmen = Control
Day = 7 subtracted from:

Level Treatmen*Day		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Control	0	10.167	1.753	5.7986	1.0000
Control	28	10.333	1.753	5.8936	1.0000
Product	0	12.500	1.753	7.1294	1.0000
Product	7	1.000	1.753	0.5704	0.9477
Product	28	5.000	1.753	2.8518	0.9999

General Linear Model: Ranked aromatics versus Treatments, Day

Factor	Type	Levels	Values
Treatmen	fixed	2	Control Product
Day	fixed	3	0 7 28

Analysis of Variance for Ranked a, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatmen	1	2.000	2.000	2.000	0.43	0.523
Day	2	364.333	364.333	182.167	39.51	0.000
Treatmen*Day	2	50.333	50.333	25.167	5.46	0.021
Error	12	55.333	55.333	4.611		
Total	17	472.000				

Unusual Observations for Ranked a

Obs	Ranked a	Fit	SE Fit	Residual	St Resid
2	17.0000	13.1667	1.2398	3.8333	2.19R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Ranked a
Comparisons with Control Level
Treatmen = Control
Day = 28 subtracted from:

Treatmen	Day	Difference of Means	Upper	------+---------+---------+---------+
Control	0	-0.17	4.221	----------*------)
Control	7	-10.33	-5.946	---------*------)
Product	0	2.17	6.554	----------*------)
Product	7	-9.33	-4.946	----------*-------)
Product	28	-5.33	-0.946	----------*------)

------+---------+---------+---------+
-12.0 -6.0 0.0 6.0

Dunnett Simultaneous Tests
Response Variable Ranked a
Comparisons with Control Level
Treatmen = Control
Day = 28 subtracted from:

Level Treatmen	Day	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Control	0	-0.17	1.753	-0.095	0.8040
Control	7	-10.33	1.753	-5.894	0.0002
Product	0	2.17	1.753	1.236	0.9900
Product	7	-9.33	1.753	-5.323	0.0004
Product	28	-5.33	1.753	-3.042	0.0196

} These are significantly less than Day 28 controls.

MICROBIOLOGICAL RESULTS

STATISTICAL ANALYSIS

COMPUTER PRINTOUTS

APPENDIX III

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                 Version 4.04                   *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 0 Control Rep#1 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	3
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 60.61
MPN CORRECTED FOR BIAS (Salama) = 55.14
SPEARMAN-KARBER ESTIMATE = 100

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 6.939
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 141.5

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*                CINCINNATI, OHIO                *
**************************************************
```

Day 0 Control Rep#2 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	0
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 00.00
MPN CORRECTED FOR BIAS (Salama) = 00.00
SPEARMAN-KARBER ESTIMATE = 0

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                 Version 4.04                   *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 0 Control Rep#3 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	2
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 36.1
MPN CORRECTED FOR BIAS (Salama) = 33.17
SPEARMAN-KARBER ESTIMATE = 68.13

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 2.668
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 94.81

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*                CINCINNATI, OHIO                *
**************************************************
```

Day 0 Treatment Rep#1 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	3
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 60.61
MPN CORRECTED FOR BIAS (Salama) = 55.14
SPEARMAN-KARBER ESTIMATE = 100

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 6.939
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 141.5

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 0 Treatment Rep#2 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	1
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 16.43
MPN CORRECTED FOR BIAS (Salama) = 15.2
SPEARMAN-KARBER ESTIMATE = 46.42

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 2.668
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 55.48

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 0 Treatment Rep#3 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	5
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 141.7
MPN CORRECTED FOR BIAS (Salama) = 123.4
SPEARMAN-KARBER ESTIMATE = 215.4

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 42.29
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 318.3

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*                CINCINNATI, OHIO                *
**************************************************
```

Day 7 Control Rep#1 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	2
.0001	6	1
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 565.5
MPN CORRECTED FOR BIAS (Salama) = 448.3
SPEARMAN-KARBER ESTIMATE = 1,000

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 207.2
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 1,333

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                Version 4.04                    *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 7 Control Rep#2 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	1
.0001	6	1
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 401.8
MPN CORRECTED FOR BIAS (Salama) = 302
SPEARMAN-KARBER ESTIMATE = 681.3

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 152.2
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 1,020

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 7 Control Rep#3 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	5
.0001	6	1
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 1,759
MPN CORRECTED FOR BIAS (Salama) = 1,507
SPEARMAN-KARBER ESTIMATE = 3,162

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 509.8
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 4,278

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                 Version 4.04                   *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 7 Treatment Rep#1 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	5
.00001	6	6
.10000E-05	6	6
.10000E-06	6	6
.10000E-07	6	5
.10000E-08	6	6

MOST PROBABLE NUMBER,,MPN (per ml) = 485,236,187
MPN CORRECTED FOR BIAS (Salama) = 346,842,093
SPEARMAN-KARBER ESTIMATE = .14678E+1

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 178,574,667
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = .123E+10

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 7 Treatment Rep#2 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	6
.00001	6	6
.10000E-05	6	6
.10000E-06	6	5
.10000E-07	6	6
.10000E-08	6	6

NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	6
.00001	6	6
.10000E-05	6	6
.10000E-06	6	5
.10000E-07	6	6
.10000E-08	6	6

```
**********************************************************
*   THE MPN ALGORITHM DID NOT CONVERGE FOR THE DATA SET.  *
*       NEITHER AN MPN OR LIMITS COULD BE CALCULATED.     *
* THE DATA SET IS NOT LIKELY UNDER THE ASSUMPTION THAT THE *
*              DATA ARE POISSON DISTRIBUTED.              *
**********************************************************
```

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*                CINCINNATI, OHIO                *
**************************************************
```

Day 7 Treatment Rep#3 IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	6
.00001	6	6
.10000E-05	6	6
.10000E-06	6	6
.10000E-07	6	6
.10000E-08	6	6

MOST PROBABLE NUMBER,,MPN (per ml) = Too numerous to count (TNTC)
MPN CORRECTED FOR BIAS (Salama) = (TNTC)
SPEARMAN-KARBER ESTIMATE = (TNTC)

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*                CINCINNATI, OHIO                *
**************************************************
```

Day 28 Rep#1 Control IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	5
.001	6	2
.0001	6	1
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 259.8
MPN CORRECTED FOR BIAS (Salama) = 209.9
SPEARMAN-KARBER ESTIMATE = 681.3

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 95.79
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 715.1

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*                CINCINNATI, OHIO                *
**************************************************
```

Day 28 Rep#2 Control IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	0
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 00.00
MPN CORRECTED FOR BIAS (Salama) = 00.00
SPEARMAN-KARBER ESTIMATE = 00.00

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                Version 4.04                    *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 28 Rep#3 Control IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	1
.001	6	0
.0001	6	0
.00001	6	0
.10000E-05	6	0
.10000E-06	6	0
.10000E-07	6	0
.10000E-08	6	0

MOST PROBABLE NUMBER,,MPN (per ml) = 16.43
MPN CORRECTED FOR BIAS (Salama) = 15.2
SPEARMAN-KARBER ESTIMATE = 46.42

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 2.668
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = 55.48

```
**************************************************
*         MOST PROBABLE NUMBER CALCULATOR        *
*                   Version 4.04                 *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*      RISK REDUCTION ENGINEERING LABORATORY     *
*                 CINCINNATI, OHIO               *
**************************************************
```

Day 28 Rep#1 Treatment IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	6
.00001	6	6
.10000E-05	6	6
.10000E-06	6	6
.10000E-07	6	3
.10000E-08	6	6

MOST PROBABLE NUMBER,,MPN (per ml) = TNTC
MPN CORRECTED FOR BIAS (Salama) = TNTC
SPEARMAN-KARBER ESTIMATE = TNTC
CANNOT BE CALCULATED

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 28 Rep#2 Treatment IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	6
.00001	6	6
.10000E-05	6	6
.10000E-06	6	6
.10000E-07	6	6
.10000E-08	6	6

MOST PROBABLE NUMBER,,MPN (per ml) = TNTC
MPN CORRECTED FOR BIAS (Salama) = TNTC
SPEARMAN-KARBER ESTIMATE = TNTC

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na

```
**************************************************
*        MOST PROBABLE NUMBER CALCULATOR         *
*                  Version 4.04                  *
* UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  *
*     RISK REDUCTION ENGINEERING LABORATORY      *
*               CINCINNATI, OHIO                 *
**************************************************
```

Day 28 Rep#3 Treatment IEP

DATA SET 1
NUMBER OF DILUTIONS = 8

VOLUME, ml	# TUBES	# POSITIVE TUBES
.01	6	6
.001	6	6
.0001	6	6
.00001	6	6
.10000E-05	6	6
.10000E-06	6	6
.10000E-07	6	6
.10000E-08	6	6

MOST PROBABLE NUMBER,,MPN (per ml) = TNTC
MPN CORRECTED FOR BIAS (Salama) = TNTC
SPEARMAN-KARBER ESTIMATE = TNTC

LOWER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na
UPPER 95% CONFIDENCE LIMIT (Cornish & Fisher) = na

GRAVAMETRIC RESULTS

STATISTICAL ANALYSIS

COMPUTER PRINTOUTS

APPENDIX IV

	C1	C2	C3
	Treatments	Day	Weights (g)
1	Control	0	0.13
2	Control	0	0.14
3	Control	0	0.15
4	Control	7	0.13
5	Control	7	0.12
6	Control	7	0.12
7	Control	28	0.14
8	Control	28	0.18
9	Control	28	0.18
10	Product	0	0.14
11	Product	0	0.13
12	Product	0	0.13
13	Product	7	0.10
14	Product	7	0.09
15	Product	7	0.12
16	Product	28	0.10
17	Product	28	0.08
18	Product	28	0.11

Two-Sample T-Test and CI: Weights, Treatments Day 0

Two-sample T for Weights

Treatmen	N	Mean	StDev	SE Mean
Control	3	0.1400	0.0100	0.0058
Product	3	0.13333	0.00577	0.0033

Difference = mu (Control) - mu (Product)
Estimate for difference: 0.00667
95% CI for difference: (-0.01455, 0.02788)
T-Test of difference = 0 (vs not =): T-Value = 1.00 P-Value = 0.391 DF = 3

Two-Sample T-Test and CI: Weights, Treatments Day 7

Two-sample T for Weights

Treatmen	N	Mean	StDev	SE Mean
Control	3	0.12333	0.00577	0.0033
Product	3	0.1033	0.0153	0.0088

Difference = mu (Control) - mu (Product)
Estimate for difference: 0.02000
95% CI for difference: (-0.02057, 0.06057)
T-Test of difference = 0 (vs not =): T-Value = 2.12 P-Value = 0.168 DF = 2

Two-Sample T-Test and CI: Weights, Treatments Day 28

Two-sample T for Weights

Treatmen	N	Mean	StDev	SE Mean
Control	3	0.1667	0.0231	0.013
Product	3	0.0967	0.0153	0.0088

Difference = mu (Control) - mu (Product)
Estimate for difference: 0.0700
95% CI for difference: (0.0191, 0.1209)
T-Test of difference = 0 (vs not =): T-Value = 4.38 P-Value = 0.022 DF = 3

RAW LABORATORY NOTES

APPENDIX V

E SUBJECT IEP PROJECT NO.

1/7/02 BIOREMEDIATION [redacted] N/A

FS-5-200

STARTED INCUBATION OF DAY 0, 7, & 28 treatments & controls @

Recvd seawater from Galveston Bay on 2/1/02. C-O-C ✓ water. Filtered seawater thru glass wool (sterile) to remove any debri. Sterile 250 ml Erlenmeyer flasks had 100 ml of filtered seawater placed into each. Buffer solⁿ for micro was prepared & sterilized. Bushnell-Haas broth was also prepared & ~~steril~~ sterilized. ~0.5 g of MNS 521 oil was pipetted from sterile pasteur pipettes into each Erlenmeyer flask. This was followed by pipetting 0.1 g (20% of oil wt) of bioremediator agent on top of oil droplet in each flask. Flasks were then placed on orbital shaker. Shaker speed was 190-200 rpm. Incubation temp was set @ 20°C. Day 0 flasks (treatment & controls) were agitated for 10 min prior to removing from orbital shaker & freezing @ -10°C for lab extractions. Prior to freezing, Day Remaining flasks were remained on orbital shaker until ~~dept~~ respective day of sacrifice.

UAB

SSED AND UNDERSTOOD

SUBJECT PROJECT NO. F5 5-200

1/02 ANS 521 oil wts added to each flask

~~TREAT~~ TREATMENT	DAY 0
Rep #1	0.52g
" #2	0.50g
" #3	0.50g
TREATMENT	DAY 7
Rep #1	0.50g
" #2	0.51g
" #3	0.51g
TREATMENT	DAY 28
Rep #1	0.51g
" #2	0.50g
" #3	0.50g

CONTROL	DAY 0
Rep #1	0.51g
" #2	0.50g
" #3	0.51g
CONTROL	DAY 7
Rep #1	0.50g
" #2	0.52g
" #3	0.51g
CONTROL	DAY 28
Rep #1	~~0.05~~ 0.52g
" #2	0.51g
" #3	0.50g

CRO
Day 0
1/8/02

1 DAY
3/15

Each flask ē 100ml seawater weighed on SARTORIUS 1264 MD ANALYTICAL SCALE, TARED & set to zero. oil then added & weighed to as close to 0.5g as possible. 20% of wt of oil (6 drops from pasteur pipette) of bioremediation agent inoculated into each treatment flask. Orbital shaker ran 10 min then stopped to remove DAY 0 treatments & controls. 0.5 ml of ~~water~~ water from each flask was drawn from each DAY 0 treatment and control & used to inoculate micro phosphate buffer solⁿ. Buffer solⁿ then made into 8 serial dilution 0.1ml of each buffer serial dilution was ~~pr~~ pipetted into 6 reps each. 20µl of No. 2 Fuel oil (sterile) was pipetted onto top of solⁿ in each well after 1.75ml ~~not~~ sterile B-H broth, followed by 0.1ml of serially diluted buffer solⁿs inoculated with water from test & control flasks. DAY 7 treatments & controls well shaken on Friday March 1, 02 and prepared as above for incubation. All incubation took place @ 20°C. (NOTE - March 22, 02 is Day 28)

VMB

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SIGNED	DATE	DATE

TE SUBJECT PROJECT NO.

Day 0 Micro Reading

Control Rep #1

~~(Rep 1)~~ Dilution

Rep #	1	2	3	4	5	6	7	8
1	−	−	−	−	−	−	−	−
2	+	−	−	−	−	−	−	−
3	+	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	+	−	−	−	−	−	−	−

Control Rep #2

Dilution #

Rep	1	2	3	4	5	6	7	8
1	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−

Control Rep #3

Dilution

Rep	1	2	3	4	5	6	7	8
1	+	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−
4	+	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−

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DATE | SUBJECT | PROJECT NO.

1 1 '22 | Cont' Micro reading Day 0

TREATMENT Rep #1

Rep	Dilution 1	2	3	4	5	6	7	8
1	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–
3	+	–	–	–	–	–	–	–
4	+	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–
6	+	–	–	–	–	–	–	–

TREATMENT Rep #2

Rep	Dilution 1	2	3	4	5	6	7	8
1	–	–	–	–	–	–	–	–
2	+	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–

TREATMENT Rep #3

Rep	Dilution 1	2	3	4	5	6	7	8
1	+	–	–	–	–	–	–	–
2	+	–	–	–	–	–	–	–
3	+	–	–	–	–	–	–	–
4	+	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–
6	+	–	–	–	–	–	–	–

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ED | DATE | SIGNED

DATE SUBJECT PROJECT NO.

,02 Micro Reading Day 7

Dilution Treatment Rep #1

Rep #	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	-	+	+	+	+	+
3	+	+	+	+	+	+	-	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+

Dilution Treatment Rep #2

Rep	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	-	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+

Dilution Treatment Rep #3

Rep	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+

~~Treatment~~ ~~Control Rep #~~

WHB

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NED DATE SIGNED

DATE SUBJECT PROJECT NO.

1/5/02 MICRO Reading Day 7

Dilution CONTROL ~~TREATMENT~~ Rep #1

Rep	1	2	3	4	5	6	7	8
1	+	+	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-
4	+	-	+	-	-	-	-	-
5	+	-	-	-	-	-	-	-
6	+	-	-	-	-	-	-	-

Control Rep# 2

Dilution

Rep	1	2	3	4	5	6	7	8
1	+	-	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-
4	+	-	+	-	-	-	-	-
5	+	-	-	-	-	-	-	-
6	+	-	-	-	-	-	-	-

CONTROL Rep#3

Rep	1	2	3	4	5	6	7	8
1	+	+	-	-	-	-	-	-
2	+	+	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-
5	+	+	+	-	-	-	-	-
6	+	+	-	-	-	-	-	-

TE SUBJECT

1/31/02

Started Day 28 Micro & Sacrificed Day 28 samples.

Day 28 treatments were removed from orbital shaker. 0.5 ml were removed from each treatment and control and used to inoculate micro titer plates. Plates to be read on 4/5/02. Fresh soln of B+H broth was sterilized and allowed to cool prior to use. 4.5 ml of Buffer soln was pipetted into sterile test tubes (4.5 ml of buffer soln). 0.5 ml of treatment or control water was pipetted into first of eight serial dilution. After inoculation, test tube was placed on vortex genie to thoroughly mix soln. 0.5 ml of mixed soln was then used to inoculate next dilution down from $10^{-1} \rightarrow 10^{-8}$. 0.1 ml of each test tube was used to inoculate microtiter plate. 20 µl of sterile No 2. fuel oil then pipetted on top of each microtiter well. Micro titer plates were then incubated @ 28° ~~@~~ °C.

1/27/28

Vials for gravimetric analysis labeled & weighed for later blowdown. Vials to be sent w/ Day 28 samples to be filled w/ 18 ml of effluent (done by LSU personnel)

Empty vial w/ caps weights for gravimetric analysis. (Day 28) 0,7

Day 28 Control Rep #1 - 18.17 g
" " #2 - 18.05 g
" " 3 - 18.11 g

Treatment Rep #1 - 18.03 g
" " 2 - 18.09 g
" " 3 - 18.16 g

Day 7 Control Rep #1 - 21.64 g
" " 2 - 21.44 g
" " 3 - 21.70 g

Treatment Rep #1 - 21.52 g
" 2 - 21.68 g
" 3 - 21.61 g

Day 0 Control Rep #1 - 21.72 g
" " 2 - 21.69 g
" " 3 - 21.63 g

Treatment Rep #1 - 21.60 g
" #2 - 21.60 g
" #3 - 21.62 g

ESSED AND UNDERSTOOD

DATE SUBJECT PROJECT NO.

1.5/02 Read Day 28 micro after 1.5 hrs reaction time Rgt catalyst was p-iodotetramethylaniline violet (100 µL) per well.

Control Day 28 Rep #1

	1	2	3	4	5	6	7	8
1	+	-	-	-	-	-	-	-
2	+	+	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	+	-	+	-	-	-	-	-
6	+	+	-	-	-	-	-	-

Control Day 28 Rep #2

	1	2	3	4	5	6	7	8
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-

Control Day 28 Rep #3

	1	2	3	4	5	6	7	8
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-

TREATMENT Day 28 Rep #1

	1	2	3	4	5	6	7	8
1	+	+	+	+	+	-	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+
4	+	+	+	+	+	-	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+

TREATMENT Day 28 Rep #2

	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+

TREATMENT Day 28 Rep #3

	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+

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~~Hotel~~ IEP Na

1/8/62 Gravimetric Analysis 10ml of each extract Day 0, 7, 28

Weights of glass vials & caps + & out cap + with 10ml extract. done by LSL-

		Initial ~~1st Blowdn~~	2nd. Blowdown		3rd Blowdn
Day 0 Control	Rep # 1	34.565	21.85		~~21.68~~ 21.82
	#2	34.46 g	21.84	21.83	21.83
wt of vial & cap	#3	34.48 g	21.80		21.78
#1 21.72					
#2 21.69					
#3 21.63					

		Initial	1st Blowdn	2nd Blowdn	3rd Blowdn
Day 0 Treatment	Rep # 1	34.43 g	21.76	21.74	21.74
	#2	34.39 g	21.89	21.73	21.73
	#3	34.47 g	21.76	21.75	21.75

wt of vial & cap #1 21.60
#2 21.61
#3 21.62

Day 7 Control — Rep # 1 Initial 34.65 g (bt/l & 10ml) 1st Blowdn 21.82 2nd 21.79 3rd 21.7

wt of vial & cap #1 21.64 — Rep #2 Initial 34.53 g 1st Blowdn 21.79 2nd. 21.78 3rd 21.78

#2 21.66 — Rep #3 " 34.45 g 1st Blowdn 21.86 2nd 21.85 3rd 21.8

#3 21.70

Day 7 Treatment — Rep # 1 Initial 34.38 g 1st 21.69 2nd 21.62 3rd 21.62

#21.52 — Rep #2 " 34.58 g 1st 21.80 2nd 21.78 · 3rd 21.77

#2 21.68 — ~~Blkep #3~~ Rep #3 " 34.53 1st 21.75 2nd 21.73 3rd 21.73

#3 21.61

SUBJECT - IEP GRAVIMETRIC PROJECT NO.

DAY 28 CONTROL

	Initial (vial + 10ml)	1st Blowdn	2nd Blowdn	3rd Blowdn
#1	30.82 g	16.41 g	16.41 g	18.40 g
#2	31.27 g	18.57 g	18.55 g	18.55 g
#3	30.99 g	18.39 g	18.37 g	18.37 g

DAY 28 TREATMENT

	(Initial vial + 10ml)	1st Blowdn	2nd Blowdn	3rd Blowdn
#1	30.94 g	18.47 g	18.41 g	18.40 g
#2	30.95 g	18.42 g	18.39 g	18.39 g
#3	30.65 g	18.24 g	18.22 g	18.22 g

ESSED AND UNDERSTOOD

ED DATE SIGNED

LITERATURE REFERENCES

AND

MSDS SHEET

APPENDIX VI

Literature References

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