

STUDY TITLE

Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the
Unicellular Green Alga, *Pseudokirchneriella subcapitata*

DATA REQUIREMENT

OPPTS 850.5400
OECD Guideline 201

AUTHOR


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STUDY INITIATION DATE

24 February 2009

STUDY COMPLETION DATE

29 September 2010

SPONSOR

American Petroleum Institute
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Washington, DC 20005

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STUDY IDENTIFICATION

ABC Study No. 64405

STATEMENT OF GLP COMPLIANCE

Compound: Naphthenic Acids

Study Title: Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

The study described in this report, with the following exceptions, was conducted in compliance with the following Good Laboratory Practice Standards:

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

U.S. Environmental Protection Agency. 1989. Toxic Substances Control Act; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792).

- 1) The test substance characterization was not conducted in accordance to the stated Good Laboratory Practices.
- 2) The latest water characterizations performed in August 2009 were not performed in accordance to the stated Good Laboratory Practices.
- 3) Analyses conducted by the University of Alberta were not conducted in accordance to the stated Good Laboratory Practices.

These were the only exceptions to the stated GLP principles and they did not adversely affect the study integrity or the interpretation of the results generated from this study.

The original raw data and the study plan were provided to the American Petroleum Institute with the final report. Copies of all data in support of this report were retained at ABC Laboratories, Inc. along with original facility records and a copy of the final report and the study plan.

[Redacted Signature] 21 SEPT 2010
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[Redacted Signature] 29 Sept 10
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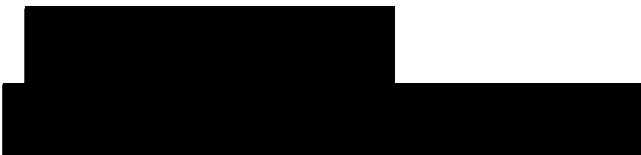
Sponsor's Representative:
[Redacted Signature] 15 November 2010
[Redacted Signature]
Date
American Petroleum Institute

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study No. 64405 entitled, "Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*," for American Petroleum Institute. The following inspections/audits were conducted on this study.

Date of Study Based Inspection	Phase Inspected	Date Reported to the Study Director	Date Reported to ABC Management
12 Oct 09	Procedure – Cell Counts	13 Oct 09	14 Oct 09
13-15 Jan 10	Raw Data & Draft Report	15 Jan 10	26 Jan 10
27 Sep 10	Final Report	27 Sep 10	27 Sep 10

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



ABC Laboratories, Inc.

29 Sep 2010
Date

STUDY PERSONNEL

Study Director

[REDACTED]

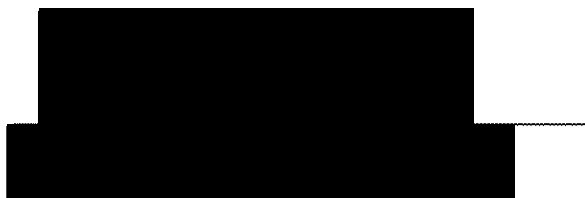
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29 Sep 10
Date

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STUDY SUMMARY

Study Sponsor:	American Petroleum Institute		
Protocol Title:	Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>		
Location of Study:	ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202 Department of Biological Sciences Z-207 Biological Sciences Centre 116 th Street and 85 th Avenue University of Alberta Edmonton, Alberta T6G 2R3 Canada		
ABC Study No.:	64405		
Test Substance:	Naphthenic Acids (CAS# 1338-24-5)		
Test Species:	<i>Pseudokirchneriella subcapitata</i>		
Source of Test Species:	University of Texas at Austin (UTEX)		
Definitive Test Dates (in-life):	October 09 to 13, 2009		
Test Duration:	96 hours		
Nominal Loading Rates:	0 (control), 2.5, 5.0, 10, 20, 40, and 80 mg naphthenic Acids/L		
72 Hour Mean Measured Concentrations:	<MQL (control), 1.69, 3.48, 7.38, 15.0, 28.9, and 44.9 mg naphthenic acids/L		
96 Hour Mean Measured Concentrations:	<MQL (control), 1.64, 3.51, 7.41, 14.8, 28.4, and 44.8 mg naphthenic acids/L		
Environmental Conditions: (in biotic replicates)	Test Solution Temperature:	23.2 to 24.2°C	
	Test Solution pH:	6.8 to 8.9	
	Photoperiod:	continuous light	
	Light Intensity:	4,357 to 4,527 lux	

Results Based on Nominal Loading Rates:

Hour	EL Type	EL Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOELR (mg Naphthenic Acids/L)
72	E _r L ₅	28.3	22.2 to 34.3	10
	E _r L ₁₀	31.1	26.3 to 35.9	
	E _r L ₂₀	34.6	31.4 to 37.7	
	E _r L ₅₀	41.3	40.3 to 42.3	
	E _r L ₉₀	54.9	44.3 to 65.5	
	E _y L ₅	9.32	7.84 to 10.8	10
	E _y L ₁₀	11.8	10.3 to 13.3	
	E _y L ₂₀	15.3	13.8 to 16.8	
	E _y L ₅₀	23.8	22.5 to 25.1	
	E _y L ₉₀	47.8	43.3 to 52.3	
96	E _r L ₅	25.6	22.9 to 28.3	10
	E _r L ₁₀	29.2	27.0 to 31.5	
	E _r L ₂₀	33.8	32.1 to 35.5	
	E _r L ₅₀	43.3	42.0 to 44.6	
	E _r L ₉₀	64.2	57.8 to 70.6	
	E _y L ₅	11.6	10.3 to 13.0	10
	E _y L ₁₀	14.1	12.8 to 15.4	
	E _y L ₂₀	17.4	16.1 to 18.6	
	E _y L ₅₀	24.8	23.6 to 25.9	
	E _y L ₉₀	43.5	40.3 to 46.6	

Results Based on Mean Measured Concentrations:

Hour	EC Type	EC Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOEC (mg Naphthenic Acids/L)
72	E _r C ₅	22.0	21.8 to 22.3	7.38
	E _r C ₁₀	23.8	23.5 to 24.1	
	E _r C ₂₀	25.8	25.5 to 26.1	
	E _r C ₅₀	29.6	29.3 to 30.0	
	E _r C ₉₀	36.9	36.5 to 37.3	
	E _y C ₅	7.64	6.47 to 8.80	7.38
	E _y C ₁₀	9.45	8.28 to 10.6	
	E _y C ₂₀	11.9	10.8 to 13.0	
	E _y C ₅₀	17.7	16.7 to 18.7	
	E _y C ₉₀	33.0	30.1 to 36.0	
96	E _r C ₅	21.2	19.7 to 22.6	7.41
	E _r C ₁₀	23.1	21.9 to 24.3	
	E _r C ₂₀	25.4	24.6 to 26.3	
	E _r C ₅₀	29.9	29.3 to 30.5	
	E _r C ₉₀	38.7	30.5 to 41.2	
	E _y C ₅	9.03	8.02 to 10.0	7.41
	E _y C ₁₀	10.8	9.79 to 11.7	
	E _y C ₂₀	13.0	12.1 to 13.9	
	E _y C ₅₀	18.1	17.3 to 18.9	
	E _y C ₉₀	30.3	28.3 to 32.4	

Note: Endpoints calculated for 72-hours are based on the 72-hour mean measured concentrations. Endpoints calculated for 96-hours are based on the 96-hour mean measured concentrations.

1.0 INTRODUCTION

The American Petroleum Institute contracted ABC Laboratories, Inc. to perform a 96-hour growth inhibition test with the freshwater green alga, *Pseudokirchneriella subcapitata*, exposed to water accommodated fraction (WAF) preparations of naphthenic acids (CAS# 1338-24-5). The primary objective of the test was to define the concentration response curve and determine the 72- and 96-hour EL_{50} and EC_{50} values of the test substance with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) under static test conditions. Reduction in algal cell density, algal growth rates, and yield in test substance treatments relative to the controls were used to evaluate the phytotoxicity of the test substance. In addition, a no-observed-effect loading rate (NOELR) and concentration (NOEC) at 72- and 96-hours was determined.

2.0 MATERIALS AND METHODS

2.1 Test and Reference Substance

A sample of the test substance, naphthenic acids (CAS# 1338-24-5); EPL P/A #1203-000 (collected from Drum #2), was received from EPL Archives, Inc. on January 20, 2009 and was stored at room temperature. An expiration date of the sample was not provided. The sample was assigned ABC reference number TS-22856. The Material Sample Safety Data Sheet (MSDS) described the test substance as an amber-colored liquid and stable under normal storage conditions. The MSDS and a profile of the physical chemical specifications of the test substance provided by the original supplier are given in [Appendix A](#). This material was used to prepare all test solutions, matrix spiking solutions, and analytical standards. All solution preparations were based on total product.

2.2 Test Species

The parent stock of *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*, was obtained from the Department of Botany, Culture Collection of Algae, University of Texas at Austin, on 30 June 2009. The parent stock was identified as *Selenastrum capricornutum*. The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing. The algal culture used for this test was three days old at test initiation and the biomass had increased exponentially (i.e., specific growth rate of 1.5 day^{-1}) during the culture period.

2.3 Test Medium

The test medium was a freshwater algal nutrient medium ([1](#)). The medium was prepared by the addition of appropriate reagent grade salts to autoclaved ABC reagent water. ABC reagent water is produced by passing reverse-osmosis water through a series of deionization tanks, a laboratory water purification system consisting of carbon, de-mineralization, and organic adsorption cartridges, and then through a $0.2\text{-}\mu\text{m}$ filter. After preparation, the medium was pH-adjusted to 7.5 ± 0.1 using 0.1 N NaOH and filtered through $0.45\text{-}\mu\text{m}$ Millipore[®] filters. Chemical

characterization of a representative sample of the base water, i.e., ABC well water used to prepare ABC reagent water is presented in [Appendix B](#).

2.4 Biological Test Methods

Test procedures followed the ABC test protocol entitled, “Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*,” with amendments ([Appendix C](#)). The protocol was designed to meet U.S. EPA guideline 850.5400 (2) and OECD guideline 201 (3). Modifications to the regulatory guidelines were made to address the testing of insoluble and complex mixtures (4, 5). This included adopting the WAF method of preparing exposure solutions, which is the preferred method when testing multi-component substances that are only partially soluble in water. By definition, the term WAF is applied to aqueous media containing only the fraction of multi-component substances that is dissolved and/or present as a stable dispersion or emulsion. A WAF equilibration study was done in advance of the toxicity tests to determine the optimum mixing time required to achieve equilibration of naphthenic acids dissolution in water. This is reported in ABC Study No. 64403 (6).

2.4.1 Range-Finding Test

A 96-hour range-finding test was conducted from August 07 to 11, 2009, at nominal loading rates of 0 (control), 10, 25, 50, and 100 mg naphthenic acids/L. One water accommodated fraction (WAF) at each loading rate was prepared by adding the appropriate amount of test substance to 2 L of dilution medium in an autoclaved clean 2-L glass carboy. The control WAF preparation consisted of dilution medium only. Each carboy contained a 2-inch Teflon-coated stir bar and was sealed with a screw cap. The WAF preparations were allowed to stir for 24 hours $\pm$ 1 hour. The stirring speed was adequate so that the vortex was ~30 to 50% of the solution depth. After the stirring period, the stirring was stopped and each preparation was allowed to settle for approximately 1 hour before collection. To collect the WAF products, the solutions were drained from the outlet of their respective aspirator bottle. The first ~100 mL of solution from each WAF was drained into a waste container. All treatments consisted of three exposure flasks. At test initiation, all replicates of the control and each test substance treatment (replicates A, B, and C) were inoculated with 1.0 mL of an algal concentrate containing approximately 1.0×10^6 cells/mL, resulting in a final density of approximately 1.0×10^4 cells/mL for each flask. At test initiation and termination, the controls and all test substance treatment solutions were clear and colorless with no visible precipitate, surface film, or undissolved test substance. The control cell density at termination was 92×10^4 cells/mL. The percent change in cell density at 96 hours, as compared to the control mean cell density, was +1, -41, -99, and -99% in the 10, 25, 50, and 100 mg naphthenic acids/L treatments, respectively ([Table 1](#)). Based on these results, a nominal concentration range of 0 (control), 2.5, 5.0, 10, 20, 40, and 80 mg naphthenic acids/L was selected for the definitive test.

2.4.2 Definitive Test

The in-life phase of the definitive test was conducted from October 09 to 13, 2009. One water accommodated fraction (WAF) at each loading rate was prepared by adding the appropriate amount of test substance to 4 L of dilution medium in an autoclaved clean 4-L glass carboy. The control WAF preparation consisted of dilution medium only. Each carboy contained a 2-inch

Teflon-coated stir bar and was sealed with a screw cap. The WAF preparations were allowed to stir for 24 hours $\pm$ 1 hour. The stirring speed was adequate so that the vortex was ~30 to 50% of the solution depth. After the stirring period, the stirring was stopped and each preparation was allowed to settle for approximately 1 hour before collection. To collect the WAF products, the solutions were drained from the outlet of their respective aspirator bottle. The first ~100 mL of solution from each WAF was drained into a waste container.

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labeled with study number, treatment, replicate, and grid position. Prior to test initiation, the flasks were cleaned and autoclaved according to ABC standard operating procedures. The control and each test substance treatment were replicated seven times (i.e., replicates A, B, C, D, E, F, and G). Each replicate (A, B, C, D, E, and F) contained 100 mL of the appropriate parent solution in 250-mL Erlenmeyer flasks. Replicates D, E, and F of each test substance treatment were prepared to provide additional analytical sample volume at 96 hours. Replicate G of the control and each test substance treatment contained 600 mL of the appropriate parent solution and was prepared in a 1-L Erlenmeyer flask and used for analytical samples at 72 hours. At test initiation, all 100 mL replicates of the control and each test substance treatment (replicates A, B, C, D, E, and F) were inoculated with 1.0 mL of an algal concentrate containing approximately 1.0×10^6 cells/mL, resulting in a final density of approximately 1.0×10^4 cells/mL for each flask. Replicate G of each treatment was inoculated with 6 mL of the prepared inoculum along with all other replicates. These 600-mL volume replicates received the same 1.0×10^4 cells/mL initial cell density as all other replicates. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, 72, and 96 hours ($\pm$ 1 hour), cell density was measured in all 100 mL replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer.

During the four-day exposure period, the flasks were randomly positioned daily using a computer-generated random number table and incubated at $24 \pm 2^\circ\text{C}$ in a temperature controlled environmental chamber under continuous cool-white fluorescent lighting. A continuous recording of environmental chamber temperature was made from one uninoculated blank flask using an electronic datalogger with thermistor probe. Light intensity was measured daily with a LI-COR Model LI-189 light meter equipped with a LI-COR photometric sensor and ranged from 4,357 to 4,527 lux. The flasks were swirled on orbital shaker tables at 100 rpm throughout the test. Temperature and pH were measured in all parent solutions prior to distribution of the solutions to the test flasks. At 72 hours, temperature and pH were measured in replicate G of the control and each test substance treatment. At 96 hours, temperature and pH were measured in replicate A of the control and each test substance treatment. All temperature and pH measurements of the test solutions were performed with a WTW Model pH 330i meter.

2.5 Analytical Test Method

Test solutions were analyzed for the concentration of naphthenic acids using Fourier transform infrared spectroscopy (FTIR). Analysis was accomplished based on the method described by Jivraj et al. (7) and developed at ABC Laboratories (6). Details of the sample collection, preparation, and method of analysis are described below.

2.5.1 Preparation of Analytical Standard and Matrix Spiking Solutions

A primary stock solution of the test substance was prepared on April 13, 2009 by weighing 10,001.0 mg of naphthenic acids into a 100-mL class A volumetric flask and bringing the flask to volume with acetonitrile for a concentration of 100 mg naphthenic acids/mL. Subsequent dilutions of this primary stock solution were prepared in acetonitrile. The primary stock and dilutions were used for quality control (QC) fortification samples during the range-finding and definitive tests. All solutions were stored at room temperature when not in use.

A primary stock solution of the test substance was prepared on March 19, 2009 by weighing 507.7 mg of naphthenic acids into a 100-mL class A volumetric flask and bringing the flask to volume with methylene chloride for a concentration of 5,080 mg naphthenic acids/L. Subsequent dilutions of this primary stock solution were prepared in methylene chloride. The dilutions were used to prepare analytical standards for this analyte. All solutions were stored at room temperature when not in use.

2.5.2 Test Solution Analysis

The concentration of total dissolved naphthenic acids was measured in test solution samples collected at 0 and 96 hours of the range-finding test and 0, 72, and 96 hours of the definitive test. Samples from freshly-prepared parent solutions were collected at 0 hours of the range-finding and definitive tests, from replicate G of the control and each test substance treatment at 72 hours of the definitive test, and from composite samples, after combining replicate solutions by treatment, at 96 hours of the range-finding and definitive tests. The analyses were completed on October 14, 2009. Control and naphthenic acids-fortified samples were also prepared for analysis at each sample period.

A volume of 500 mL was collected and transferred to 1,000-mL separatory funnel. Each sample was acidified with concentrated sulfuric acid to a pH level of 2.5 ± 0.1 . A 100-mL volume of methylene chloride was added to each sample and the samples were shaken to mix. After approximately one minute of shaking, the sample phases were allowed to separate. The methylene chloride (lower layer) was filtered through anhydrous sodium sulfate and collected in a 500-mL flat-bottomed flask. The remaining aqueous sample was extracted a second time following the same procedure. The methylene chloride phase from the second extraction was filtered into the original flask containing the first filtrate. Each sample was then evaporated to dryness using a rotary evaporator and quantitatively transferred to 15-mL culture tubes using two separate 5-mL aliquots of methylene chloride. The samples were then evaporated to dryness under a gentle stream of nitrogen and then reconstituted with an appropriate volume of methylene chloride. Dilutions were made using methylene chloride, if necessary, to produce an analyte concentration that was within the range of the standard curve. The samples were vialled and analyzed by FTIR. QC fortifications were prepared in a similar manner after control medium had been fortified with the test substance.

2.5.3 Instrumentation Conditions

Sample analysis was performed using a FTIR system equipped with the following analytical parameters:

Manufacturer: Thermo Nicolet
 Model: Avatar 360
 Software: Omnic 32
 IR Cell: Thermo Scientific, KBr 1.0 mm sealed cell
 Cell Holder: Thermo Scientific
 Dry Nitrogen Gas Used to Protect the IR Cell Between Runs: Yes
 Scan Times: 64
 Scan Range: 4000-400 cm^{-1}
 Scan Model: Absorbance
 Resolution: 4 cm^{-1}
 Wave Number of Interest: 1743 cm^{-1}
 Solvent Used for Background Collection: Methylene chloride

2.5.4 Calculations

Naphthenic acid concentrations were determined directly from the standard curve by the following equation:

$$\frac{\left(\begin{array}{c} \mu\text{g/L or mg/L equivalents for} \\ \text{test substance from standard} \\ \text{curve equation} \end{array} \right) \left(\begin{array}{c} \text{sample volume} \\ \text{in mL for} \\ \text{chromatography} \end{array} \right)}{(\text{sample volume in mL before preparation})} = \frac{\mu\text{g/L or}}{\text{mg/L}} = \frac{\text{ppb or}}{\text{ppm}}$$

The standard curve equation is of the form: $y = mx + b$

where:

y = peak response
 m = slope of the standard curve
 x = mg/L
 b = y-intercept

Example calculation for the 10 mg/L nominal loading rate WAF sample at 0 hours:

Standard Curve: $y = 0.000158837x + 0.007289944$

Sample Peak Response: 0.0232

Concentration from standard curve: 100.166 mg/L

Volume for Analysis: 40 mL

Sample Volume: 500 mL

The concentration of naphthenic acid in the sample was calculated by the following equation:

$$\frac{(100.166 \text{ mg naphthenic acid})(40 \text{ mL})}{500 \text{ mL}} = 8.01 \text{ mg/L}$$

The minimum quantifiable limit (MQL) was determined from the following equation:

$$\frac{\left(\begin{array}{c} \text{low standard} \\ \text{concentration mg/L} \end{array} \right) \left(\begin{array}{c} \text{analysis} \\ \text{volume (mL)} \end{array} \right)}{\left(\begin{array}{c} \text{sample} \\ \text{volume (mL)} \end{array} \right)} = \text{MQL expressed as mg/L}$$

Lowest standard concentration: 75.0 µg/mL

Analysis volume: 4 mL

Sample volume: 500 mL

therefore:

$$\text{MQL} = \frac{(75.0 \text{ mg/L})(4 \text{ mL})}{(500 \text{ mL})} = 0.600 \text{ mg/L}$$

2.6 Statistical Analysis

All statistical analyses were performed with SAS software (Version 9.1).

2.6.1 NOEC Estimates

The NOELR and NOEC values, based on growth rate and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a two-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was the mean for the growth parameter was reduced or enhanced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (i.e., $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (i.e., $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. A non-parametric analysis was performed on all growth rate and yield data at 24, 48, 72, and 96 hours.

2.6.2 Percent Inhibition Calculations and EC Estimates

The specific growth rate in each treatment was calculated for each period, i.e., 0 to 24, 0 to 48, 0 to 72, and 0 to 96 hours using the following equation:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

where:

μ = average specific growth rate
 N_n = cell density at second time point
 N_1 = cell density at 0-hour time point
 t_n = second time point
 t_1 = 0-hour time point

Yield in each treatment was calculated for each period, i.e., 24, 48, 72, and 96 hours using the following equation:

$$\text{Yield} = \text{Biomass (cell counts) at hour N} - \text{Biomass (cell counts) at 0 hour}$$

where:

N = time point

The E_rL_{50} , E_yL_{50} , E_rC_{50} , and E_yC_{50} estimates were calculated using a logistic (sigmoid-shaped) model fit to the data with percent inhibition as the dependent variable and concentration as the independent variable. The percent inhibition was calculated based on specific growth rate and yield using the following formula.

$$\frac{\text{Control Mean} - \text{Treatment}}{\text{Control Mean}} \times 100\% = \% \text{ Inhibition}$$

The model used to describe the response to increasing test substance concentrations was the four-parameter logistic model with two parameters fixed; the minimum percent inhibition (A) at 0%, and the maximum percent inhibition (D) at 100%. The model was fit only in instances where the mean percent inhibition at the highest test substance treatment was greater than 45%. The model is:

$$y = D + \left[\frac{(A - D)}{(1 + [(x^B) \times (EC_{50}^{-B})])} \right]$$

where:

y = inhibition
x = test concentration
B = slope
 EC_{50} = concentration corresponding to a response halfway between the minimum and maximum, 50% inhibition in this case

A nonlinear modeling procedure was used to estimate the slope (B) and EC values. The distribution of x hat method was used to estimate the 95% confidence limits.

2.7 Characterization and Stability of Naphthenic Acids WAFs by Analysis of Z-number and Carbon Number Distribution

As part of the characterization of naphthenic acids in the WAF solutions, Dr. Phillip M. Fedorak (Department of Biological Sciences, University of Alberta, Edmonton, Alberta Canada) was retained by the Study Sponsor to perform Gas Chromatography-Mass Spectrometry (GC-MS) analysis of a representative WAF preparation. At the initiation of the definitive test, samples of the Day 0 WAF solutions were collected and shipped to the University of Alberta, Department of Biological Sciences for analyses.

While not a quantitative technique as with the FTIR analysis, the GC-MS analyses allowed the discrimination of the naphthenic acids in the WAFs into relative abundances of each ion corresponding to the general formula for naphthenic acids, $C_nH_{2n+Z}O_2$, where n is the carbon number and Z is zero or a negative even number defining the hydrogen deficiency due to cyclization. Although no analytical method exists whereby each individual naphthenic acid molecule is identified, the GC-MS method results in a distribution of families of molecules having similar carbon numbers and Z -numbers. Details of these analyses are provided in [Appendix H](#).

3.0 RESULTS AND DISCUSSION

3.1 Analytical Results

3.1.1 FTIR Analyses - Range-Finding Test

Measured concentrations of naphthenic acids in the test substance treatment solutions at test initiation were 5.80, 16.7, 31.9, and 45.4 mg naphthenic acids/L, which represented recoveries of 45 to 67% of the nominal loading rates. The measured concentrations in test substance treatment solutions at 96 hours were 4.82, 14.5, 29.1, 38.5 mg naphthenic acids/L, which represented recoveries of 39 to 58% of the nominal loading rates and 83 to 91% of the measured concentrations at initiation. Recoveries from QC fortifications ranged from 87 to 94% of the nominal concentrations. The analytical results are summarized in ([Table 2](#)).

3.1.2 FTIR Analyses - Definitive Test

Naphthenic acid concentrations were measured in test solutions at test 0, 72, and 96 hours of the definitive test. Measured concentrations of naphthenic acids in the test solutions at test initiation were 1.83, 3.86, 8.01, 16.0, 31.4, and 47.4 mg naphthenic acids/L, which represented recoveries of 59 to 80% of the nominal loading rates ([Table 3](#)). The measured concentrations in test solutions at 72 hours were 1.54, 3.10, 6.75, 13.9, 26.4, and 42.3 mg naphthenic acids/L, representing 53 to 70% of the nominal loading rates and 80 to 89% of the initial measured concentrations. Mean measured concentrations at 72 hours were 1.69, 3.48, 7.38, 15.0, 28.9, and 44.9 mg naphthenic acids/L, which represent 56 to 75% of the nominal loading rates and 90 to 95% of the initial measured concentrations. The measured concentrations in test solutions at 96 hours were 1.55, 3.56, 7.47, 14.5, 27.4, and 44.6 mg naphthenic acids/L, which represented recoveries of 56 to 75% of the nominal loading rates and 85 to 94% of the initial measured concentrations. Mean measured concentrations at 96 hours were 1.64, 3.51, 7.41, 14.8, 28.4, and

44.8 mg naphthenic acids/L, which represent 56 to 74% of the nominal loading rates and 90 to 95% of the initial measured concentrations ([Table 3](#)). No residues of naphthenic acids were detected in the control solutions above the MQL of 0.600 mg naphthenic acids/L. Recoveries from QC fortifications ranged from 90 to 104% of the nominal concentrations. The biological response results are based upon the nominal loading rates and mean measured concentrations. Endpoints calculated for time periods ≤ 72 -hours are based on the 72-hour mean measured concentrations. Endpoints calculated for 96-hours are based on the 96-hour mean measured concentrations.

3.1.3 GC/MS Analyses

Results of the analysis of Z-number and C-number families indicated a predominance of naphthenic acids contained 10 to 16 carbon atoms. Approximately 81-94% of the dissolved constituents fell within this range of carbon numbers. The dissolved fractions also showed a prevalence of one and two ring naphthenic acids isomers. These isomers made up approximately 70-83% of the dissolved fraction. Typically, the third highest group of naphthenic acids was the acyclic carboxylic acids. The detailed report of these analyses is presented in [Appendix H](#).

3.2 Biological Results

After 72 hours of exposure, mean cell density in the control was 47×10^4 cells/mL, or 47 times the initial nominal cell density ([Table 4](#)). The mean cell density in the naphthenic acids treatments at 72 hours ranged from a low of 1.0×10^4 cells/mL at the nominal loading rate of 80 mg naphthenic acids/L to a high of 47×10^4 cells/mL at the nominal loading rate of 2.5 mg naphthenic acids/L. After 96 hours of exposure, mean cell density in the control was 154×10^4 cells/mL, or 154 times the initial nominal cell density ([Table 4](#)). The mean cell density in the naphthenic acids treatments at 96 hours ranged from a low of 1.2×10^4 cells/mL at the nominal loading rate of 80 mg naphthenic acids/L to a high of 151×10^4 cells/mL at the nominal loading rate of 5.0 mg naphthenic acids/L. Percent change in algal growth at 96 hours, as compared to the control, ranged from -99% at the nominal loading rate of 80 mg naphthenic acids/L to -2% at the nominal loading rate of 5.0 mg naphthenic acids/L. The cell density values for individual replicate flasks are presented in [Appendix D](#). Growth curves for the controls and naphthenic acids treatments are presented in [Figure 1](#). All algal cells appeared normal with no unusual cell shapes, color differences, flocculation, adherence of algae to test chambers, or aggregation of algal cells. A determination of algistatic from algicidal effects was not performed due to no test substance treatment being maximally inhibited (i.e., cell numbers in a treatment being less than or equal to the initial cell density at termination).

The mean growth rate values, based on growth rate from time zero, for the controls and all test substance treatments are presented in [Table 5](#). Growth rate values for individual replicate flasks are presented in [Appendix E](#). Percent change in growth rate from time zero to 96 hours, as compared to the control, ranged from -97% at the concentration of 80 mg naphthenic acids/L to -1% at the concentrations of 2.5, 5.0, and 10 mg naphthenic acids/L ([Figure 2](#)). The NOELR at 96 hours was 10 mg naphthenic acids/L (NOEC 7.41 mg naphthenic acids/L), based on the lack of a statistically significant reduction in growth rate from time zero at this and lower test substance treatments. Based on growth rate, the 72-hour E_rL_{50} was estimated to be 41.3 mg naphthenic acids/L, with 95% confidence limits of 40.3 and 42.3 mg naphthenic acids/L. Based

on growth rate, the 72-hour E_rC_{50} was estimated to be 29.6 mg naphthenic acids/L, with 95% confidence limits of 29.3 and 30.0 mg naphthenic acids/L. Based on growth rate, the 96-hour E_rL_{50} was estimated to be 43.3 mg naphthenic acids/L, with 95% confidence limits of 42.0 and 44.6 mg naphthenic acids/L. Based on growth rate, the 96-hour E_rC_{50} was estimated to be 29.9 mg naphthenic acids/L, with 95% confidence limits of 29.3 and 30.5 mg naphthenic acids/L. All results of the statistical evaluations with growth rate and the nominal concentrations are presented in [Table 7](#) and [Table 8](#). The coefficient of variation of average specific growth rates during the whole test period in control replicates was 2%. The mean coefficient of variation in growth rate at 72 hours between adjacent time periods was 10% for the control replicates ([Appendix F](#)). The mean coefficient of variation in growth rate at 96 hours between adjacent time periods was 10% for the control replicates ([Appendix F](#)).

The mean yield values for the control and all test substance treatments are presented in [Table 6](#). Yield values for individual replicate flasks are presented in [Appendix G](#). Percent change in yield at 96 hours, as compared to the control, ranged from -100% at the concentration of 80 mg naphthenic acids/L to -2% at the concentration of 5.0 mg naphthenic acids/L ([Figure 3](#)). The NOELR at 96 hours was 10 mg naphthenic acids/L (NOEC 7.41 mg naphthenic acids/L), based on the lack of a statistically significant reduction in growth rate from time zero at this and lower test substance treatments. Based on yield, the 72-hour E_yL_{50} was estimated to be 23.8 mg naphthenic acids/L, with 95% confidence limits of 22.5 and 25.1 mg naphthenic acids/L. Based on yield, the 72-hour E_yC_{50} was estimated to be 17.7 mg naphthenic acids/L, with 95% confidence limits of 16.7 and 18.7 mg naphthenic acids/L. Based on yield, the 96-hour E_yL_{50} was estimated to be 24.8 mg naphthenic acids/L, with 95% confidence limits of 23.6 and 25.9 mg naphthenic acids/L. Based on yield, the 96-hour E_yC_{50} was estimated to be 18.1 mg naphthenic acids/L, with 95% confidence limits of 17.3 and 18.9 mg naphthenic acids/L. All results of the statistical evaluations with yield and the nominal concentrations are presented in [Table 7](#) and [Table 8](#).

3.3 Water Quality

All water quality parameters remained within acceptable limits throughout the 96 hour exposure ([Table 9](#)). Test solution temperature, measured at 0, 72 and 96 hours, ranged from 23.2 to 24.2°C. Test solution pH ranged from 6.9 to 7.6 at 0 hour and ranged from 7.6 to 8.9 in biological replicates at 96 hours. At test initiation and during the exposure, all test solutions appeared clear with no color associated with the test substance, and with no visible precipitates, surface films, or undissolved test substance.

4.0 CONCLUSIONS

The test acceptability criteria were met for this study. The number of algal cells in the control at test termination was greater than 16 times the number initially inoculated to verify logarithmic phase growth. The coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7%. Since the study was not conducted as a limit test, there were at least one test concentration exhibiting less than 50% decrease in growth and one concentration exhibiting greater than 50% decrease in growth relative to the control. The pH in the control did not increase more than 1.5 units during the study. This study is classified as

acceptable and satisfies the guideline requirement for a growth inhibition test with *Pseudokirchneriella subcapitata*.

Results Based on Nominal Loading Rates:

Hour	EL Type	EL Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOELR (mg Naphthenic Acids/L)
72	E _r L ₅	28.3	22.2 to 34.3	10
	E _r L ₁₀	31.1	26.3 to 35.9	
	E _r L ₂₀	34.6	31.4 to 37.7	
	E _r L ₅₀	41.3	40.3 to 42.3	
	E _r L ₉₀	54.9	44.3 to 65.5	
	E _y L ₅	9.32	7.84 to 10.8	10
	E _y L ₁₀	11.8	10.3 to 13.3	
	E _y L ₂₀	15.3	13.8 to 16.8	
	E _y L ₅₀	23.8	22.5 to 25.1	
	E _y L ₉₀	47.8	43.3 to 52.3	
96	E _r L ₅	25.6	22.9 to 28.3	10
	E _r L ₁₀	29.2	27.0 to 31.5	
	E _r L ₂₀	33.8	32.1 to 35.5	
	E _r L ₅₀	43.3	42.0 to 44.6	
	E _r L ₉₀	64.2	57.8 to 70.6	
	E _y L ₅	11.6	10.3 to 13.0	10
	E _y L ₁₀	14.1	12.8 to 15.4	
	E _y L ₂₀	17.4	16.1 to 18.6	
	E _y L ₅₀	24.8	23.6 to 25.9	
	E _y L ₉₀	43.5	40.3 to 46.6	

Results Based on Mean Measured Concentrations:

Hour	EC Type	EC Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOEC (mg Naphthenic Acids/L)
72	E _r C ₅	22.0	21.8 to 22.3	7.38
	E _r C ₁₀	23.8	23.5 to 24.1	
	E _r C ₂₀	25.8	25.5 to 26.1	
	E _r C ₅₀	29.6	29.3 to 30.0	
	E _r C ₉₀	36.9	36.5 to 37.3	
	E _y C ₅	7.64	6.47 to 8.80	7.38
	E _y C ₁₀	9.45	8.28 to 10.6	
	E _y C ₂₀	11.9	10.8 to 13.0	
	E _y C ₅₀	17.7	16.7 to 18.7	
	E _y C ₉₀	33.0	30.1 to 36.0	
96	E _r C ₅	21.2	19.7 to 22.6	7.41
	E _r C ₁₀	23.1	21.9 to 24.3	
	E _r C ₂₀	25.4	24.6 to 26.3	
	E _r C ₅₀	29.9	29.3 to 30.5	
	E _r C ₉₀	38.7	30.5 to 41.2	
	E _y C ₅	9.03	8.02 to 10.0	7.41
	E _y C ₁₀	10.8	9.79 to 11.7	
	E _y C ₂₀	13.0	12.1 to 13.9	
	E _y C ₅₀	18.1	17.3 to 18.9	
	E _y C ₉₀	30.3	28.3 to 32.4	

Note: Endpoints calculated for 72-hours are based on the 72-hour mean measured concentrations. Endpoints calculated for 96-hours are based on the 96-hour mean measured concentrations.

PROTOCOL DEVIATIONS

None.

REFERENCES

1. American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM E1218-97a. 14pp.
2. U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, OPPTS 850.5400, Algal Toxicity, Tiers I and II, EPA712-C-96-164, 9 pp.
3. Organization for Economic Cooperation and Development (OECD). March 23, 2006. OECD Guidelines for Testing of Chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guideline No. 201.
4. Organization for Economic Cooperation and Development (OECD). 2000. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Series on Testing and Assessment, No. 23. ENV/JM/MONO(2000)6, OECD, Paris, France.
5. Girling, A.E., F.G. Whale, and D.M.M. Adema. 1994. A Guideline Supplement for Determining the Aquatic Toxicity of Poorly Water-Soluble Complex Mixtures Using Water-Accommodated Fractions. Chemosphere 29(12):2645-2649
6. Leak, A.T. 2010. Validation of Test Solution Preparations and Analytical Methods for Use in the Determination of Naphthenic Acids in Various Media Used in Environmental Toxicity Studies. ABC Laboratories, Inc. Study Number 64403.
7. Jivraj, M.N., M.D. MacKinnon, and B. Fung. 1991. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude Canada Ltd. Research Department. Edmonton, AB. September 1991.

Table 1. Biological Responses During the Range-Finding Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata* Exposed to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	96-Hr Cell Counts (cells/mL $\times 10^4$)		Percent Change as Compared to Control Mean Cell Density
	Replicate Mean	Treatment Mean	
0 (control) A	84	92	Not applicable
0 (control) B	105		
0 (control) C	88		
10 A	112	93	+1
10 B	83		
10 C	85		
25 A	53	54	-41
25 B	60		
25 C	50		
50 A	0.67	0.71	-99
50 B	0.67		
50 C	0.78		
100 A	0.78	0.67	-99
100 B	0.56		
100 C	0.67		

Table 2. Measured Concentrations of Naphthenic Acids During the Range-Finding Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*

Nominal WAF Loading Rate (mg naphthenic acids/L)	Measured Concentration of Naphthenic Acids in mg/L (Percent Nominal)			
	0-Hour	96-Hours	96-Hours Percent of 0-Hour	Mean
0 (control)	< MQL ^a	< MQL ^a	NA	< MQL ^a
10	5.80 (58)	4.82 (48)	83%	5.31 (53)
25	16.7 (67)	14.5 (58)	87%	15.6 (62)
50	31.9 (64)	29.1 (58)	91%	30.5 (61)
100	45.4 (45)	38.5 (39)	85%	42.0 (84)
<u>QC Fortification Spikes (% Recovery)</u>				
Low Spike (5.0)	4.69 (94)	4.36 (87)	---	---
High Spike (110)	100 (91)	95.9 (87)	---	---

^a Minimum Quantifiable Limit (MQL) = 0.600 mg/L.

Table 3. Measured Concentrations of Naphthenic Acids in Test Solutions During the Definitive 96-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*

Nominal WAF Loading Rate (mg naphthenic acids/L)	Measured Concentration (Percent Nominal/0 Hour)				
	0 Hour	72 Hour	72 Hour Mean	96 Hour	96 Hour Mean
Control	< MQL ^a	< MQL ^a	NA	< MQL ^a	NA
2.5	1.83 (73/---)	1.54 (62/84)	1.69 (68/92)	1.55 (62/85)	1.64 (66/90)
5.0	3.86 (77/---)	3.10 (62/80)	3.48 (70/90)	3.56 (71/92)	3.51 (70/91)
10	8.01 (80/---)	6.75 (68/84)	7.38 (74/92)	7.47 (75/93)	7.41 (74/93)
20	16.0 (80/---)	13.9 (70/87)	15.0 (75/94)	14.5 (73/91)	14.8 (74/93)
40	31.4 (79/---)	26.4 (66/84)	28.9 (72/92)	27.4 (69/87)	28.4 (71/90)
80	47.4 (59/---)	42.3 (53/89)	44.9 (56/95)	44.6 (56/94)	44.8 (56/95)
QC Fortification Spike Recoveries (Percent Nominal)					
2.00 (Low Spike)	1.86 (93)	1.79 (90)	---	1.80 (90)	---
90 (High Spike)	93.5 (104)	92.6 (103)	---	93.9 (104)	---

^a Minimum Quantifiable Limit (MQL) = 0.600 mg Naphthenic Acids/L

Table 4. Cell Density Values for *Pseudokirchneriella subcapitata* During a Definitive 96-Hour Exposure to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	Mean Cell Density (cells/mL x 10 ⁴) ^a				% Change ^b
	24 Hours	48 Hours	72 Hours	96 Hours	96 Hours
Control	3.3	13	47	154	---
2.5	3.3	12	47	147	-5
5.0	3.3	13	45	151	-2
10	3.1	12	40	149	-3
20	2.9	7.5	30	108	-30
40	1.6	3.4	8.7	22	-86
80	1.2	1.4	1.0	1.2	-99

^a Mean cell density calculated using the individual replicate densities presented in [Appendix D](#).

^b Percent change as compared to the control was determined at 96 hours using the following equation:

$$\% \text{ change} = (((\text{treatment cells/mL}) - (\text{control cells/mL})) / \text{control cells/mL}) \times 100$$

Note: Target inoculation cell density was 1.0×10^4 cells/mL.

Table 5. Growth Rate Values From Time Zero for *Pseudokirchneriella subcapitata* During a Definitive 96-Hour Exposure to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	Mean Growth Rate (cells/mL/hour) ^a				% Change ^b
	0-24 Hours	0-48 Hours	0-72 Hours	0-96 Hours (%CV)	96 Hours
Control	0.0490	0.0537	0.0534	0.0525 (2)	---
2.5	0.0501	0.0523	0.0534	0.0520 (1)	-1
5.0	0.0494	0.0533	0.0528	0.0522 (1)	-1
10	0.0470	0.0511	0.0511	0.0521 (2)	-1
20	0.0438	0.0418*	0.0473*	0.0487* (3)	-7
40	0.0186*	0.0255*	0.0300*	0.0320* (4)	-39
80	0.00629*	0.00742*	0.000305*	0.00163* (173)	-97

^a Mean growth rate values calculated using the individual replicate growth rates presented in [Appendix E](#). Values are rounded to three significant figures.

^b Percent change as compared to the control was determined at 96 hours using the following equation:

$$\% \text{ change} = (((\text{treatment growth rate}) - (\text{control growth rate})) / \text{control growth rate}) \times 100$$

* Significant reduction in growth rate as compared to the control (Dunnett's test, $p = 0.05$).

Table 6. Yield Values for *Pseudokirchneriella subcapitata* During a Definitive 96-Hour Exposure to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	Yield (cells/mL $\times 10^4$) ^a				% Change ^b
	24 Hours	48 Hours	72 Hours	96 Hours (%CV)	96 Hours
Control	2.25	12.2	46.2	153 (9)	---
2.5	2.33	11.3	45.7	146 (6)	-5
5.0	2.30	12.0	44.0	150 (6)	-2
10	2.13	10.7	39.0	148 (11)	-3
20	1.87	6.47*	29.3*	107* (15)	-30
40	0.567*	2.40*	7.73*	20.7* (14)	-86
80	0.167*	0.433*	0.0300*	0.200* (173)	-100

^a Mean yield calculated using the individual replicate yield presented in [Appendix G](#). Yield calculated as the biomass at the end of the exposure period minus the starting biomass (i.e., target inoculation cell density of 1.0×10^4 cells/mL). Values are rounded to three significant figures.

^b Percent change as compared to the control was determined at 96 hours using the following equation:

$$\% \text{ change} = (((\text{treatment yield}) - (\text{control yield})) / \text{control yield}) \times 100$$

* Significant reduction in yield as compared to the control (Dunnett's test, $p = 0.05$).

Table 7. Summary of EL and NOELR Estimates, Based on Nominal Loading Rates, for *Pseudokirchneriella subcapitata* Exposed to Naphthenic Acids

Hour	EL Type	EL Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOELR (mg Naphthenic Acids/L)
24	E _r L ₅	11.4	7.16 to 15.7	20
	E _r L ₁₀	15.2	10.6 to 19.7	
	E _r L ₂₀	20.6	16.1 to 25.2	
	E _r L ₅₀	34.9	31.1 to 38.7	
	E _r L ₉₀	>80	Not Statistically Sound	
	E _y L ₅	12.4	8.54 to 16.3	20
	E _y L ₁₀	15.5	11.6 to 19.4	
	E _y L ₂₀	19.7	16.0 to 23.4	
	E _y L ₅₀	29.7	26.8 to 32.7	
	E _y L ₉₀	57.2	48.7 to 65.7	
48	E _r L ₅	10.4	8.69 to 12.2	10
	E _r L ₁₀	14.4	12.5 to 16.4	
	E _r L ₂₀	20.5	18.5 to 22.5	
	E _r L ₅₀	37.4	35.6 to 39.2	
	E _r L ₉₀	>80	Not Statistically Sound	
	E _y L ₅	6.10	4.66 to 7.54	10
	E _y L ₁₀	8.39	6.81 to 9.98	
	E _y L ₂₀	11.9	10.2 to 13.5	
	E _y L ₅₀	21.5	19.9 to 23.0	
	E _y L ₉₀	54.9	47.6 to 62.1	

Table 7. Summary of EL and NOELR Estimates, Based on Nominal Loading Rates, for *Pseudokirchneriella subcapitata* Exposed to Naphthenic Acids (continued)

Hour	EL Type	EL Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOELR (mg Naphthenic Acids/L)
72	E _r L ₅	28.3	22.2 to 34.3	10
	E _r L ₁₀	31.1	26.3 to 35.9	
	E _r L ₂₀	34.6	31.4 to 37.7	
	E _r L ₅₀	41.3	40.3 to 42.3	
	E _r L ₉₀	54.9	44.3 to 65.5	
	E _y L ₅	9.32	7.84 to 10.8	10
	E _y L ₁₀	11.8	10.3 to 13.3	
	E _y L ₂₀	15.3	13.8 to 16.8	
	E _y L ₅₀	23.8	22.5 to 25.1	
	E _y L ₉₀	47.8	43.3 to 52.3	
96	E _r L ₅	25.6	22.9 to 28.3	10
	E _r L ₁₀	29.2	27.0 to 31.5	
	E _r L ₂₀	33.8	32.1 to 35.5	
	E _r L ₅₀	43.3	42.0 to 44.6	
	E _r L ₉₀	64.2	57.8 to 70.6	
	E _y L ₅	11.6	10.3 to 13.0	10
	E _y L ₁₀	14.1	12.8 to 15.4	
	E _y L ₂₀	17.4	16.1 to 18.6	
	E _y L ₅₀	24.8	23.6 to 25.9	
	E _y L ₉₀	43.5	40.3 to 46.6	

Table 8. Summary of EC and NOEC Estimates, Based on Mean Measured Concentrations, for *Pseudokirchneriella subcapitata* Exposed to Naphthenic Acids

Hour	EC Type	EC Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOEC (mg Naphthenic Acids/L)
24	E _r C ₅	10.8	7.77 to 14.0	15.0
	E _r C ₁₀	13.5	10.4 to 16.6	
	E _r C ₂₀	17.0	14.1 to 19.9	
	E _r C ₅₀	25.3	23.3 to 27.4	
	E _r C ₉₀	>44.9	Not Statistically Sound	
	E _y C ₅	10.2	7.77 to 12.6	15.0
	E _y C ₁₀	12.4	9.98 to 14.8	
	E _y C ₂₀	15.3	13.0 to 17.6	
	E _y C ₅₀	22.0	20.2 to 23.8	
	E _y C ₉₀	39.0	35.1 to 42.9	
48	E _r C ₅	11.5	9.42 to 13.6	7.38
	E _r C ₁₀	14.3	12.3 to 16.4	
	E _r C ₂₀	18.2	16.2 to 20.1	
	E _r C ₅₀	27.3	26.0 to 28.6	
	E _r C ₉₀	>44.9	Not Statistically Sound	
	E _y C ₅	5.13	3.98 to 6.29	7.38
	E _y C ₁₀	6.85	5.61 to 8.08	
	E _y C ₂₀	9.35	8.08 to 10.6	
	E _y C ₅₀	15.9	14.8 to 17.1	
	E _y C ₉₀	37.2	32.7 to 41.6	

Note: Endpoints calculated for time periods ≤72-hours are based on the 72-hour mean measured concentrations.

Table 8. Summary of EC and NOEC Estimates, Based on Initial Measured Concentrations, for *Pseudokirchneriella subcapitata* Exposed to Naphthenic Acids (continued)

Hour	EC Type	EC Value (mg Naphthenic Acids/L)	95% Confidence Limits (mg Naphthenic Acids/L)	NOEC (mg Naphthenic Acids/L)
72	E _r C ₅	22.0	21.8 to 22.3	7.38
	E _r C ₁₀	23.8	23.5 to 24.1	
	E _r C ₂₀	25.8	25.5 to 26.1	
	E _r C ₅₀	29.6	29.3 to 30.0	
	E _r C ₉₀	36.9	36.5 to 37.3	
	E _y C ₅	7.64	6.47 to 8.80	7.38
	E _y C ₁₀	9.45	8.28 to 10.6	
	E _y C ₂₀	11.9	10.8 to 13.0	
	E _y C ₅₀	17.7	16.7 to 18.7	
	E _y C ₉₀	33.0	30.1 to 36.0	
96	E _r C ₅	21.2	19.7 to 22.6	7.41
	E _r C ₁₀	23.1	21.9 to 24.3	
	E _r C ₂₀	25.4	24.6 to 26.3	
	E _r C ₅₀	29.9	29.3 to 30.5	
	E _r C ₉₀	38.7	30.5 to 41.2	
	E _y C ₅	9.03	8.02 to 10.0	7.41
	E _y C ₁₀	10.8	9.79 to 11.7	
	E _y C ₂₀	13.0	12.1 to 13.9	
	E _y C ₅₀	18.1	17.3 to 18.9	
	E _y C ₉₀	30.3	28.3 to 32.4	

Note: Endpoints calculated for time periods ≤ 72 -hours are based on the 72-hour mean measured concentrations. Endpoints calculated for 96-hours are based on the 96-hour mean measured concentrations.

Table 9. Test Solution Temperature and pH Measurements During a 96-Hour Exposure of *Pseudokirchneriella subcapitata* to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	0 Hour ^a		72 Hours ^b		96 Hours ^c	
	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH
Control	23.8	7.6	23.7	8.4	23.8	8.8
2.5	23.7	7.6	24.1	8.7	23.2	8.8
5.0	23.7	7.6	24.2	8.6	23.5	8.9
10	23.9	7.6	24.0	8.6	23.7	8.9
20	24.2	7.5	24.1	7.7	23.7	8.4
40	24.1	7.4	23.8	7.4	23.7	7.8
80	23.7	6.9	23.9	6.8	23.4	7.6

^a Measurements taken from parent solutions.

^b Measurements taken from replicate G test flasks.

^c Measurements taken from replicate A test flasks.

Figure 1. Growth Curves for *Pseudokirchneriella subcapitata* During a 96-Hour Exposure to Naphthenic Acids

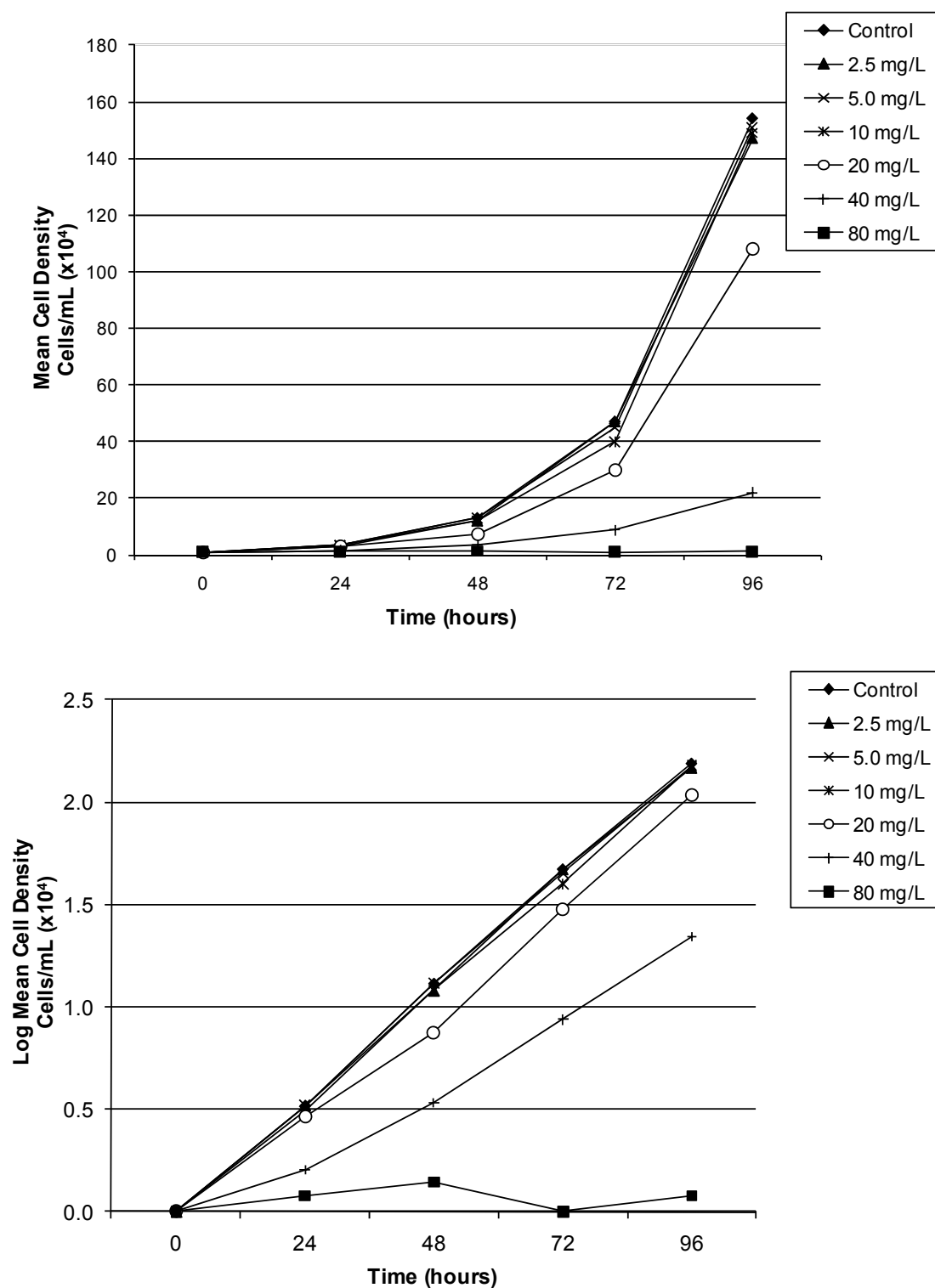


Figure 2. Plot of Percent Inhibition of *Pseudokirchneriella subcapitata* Growth Rate as Compared to the Log Concentration after 96-Hour Exposure to Naphthenic Acids

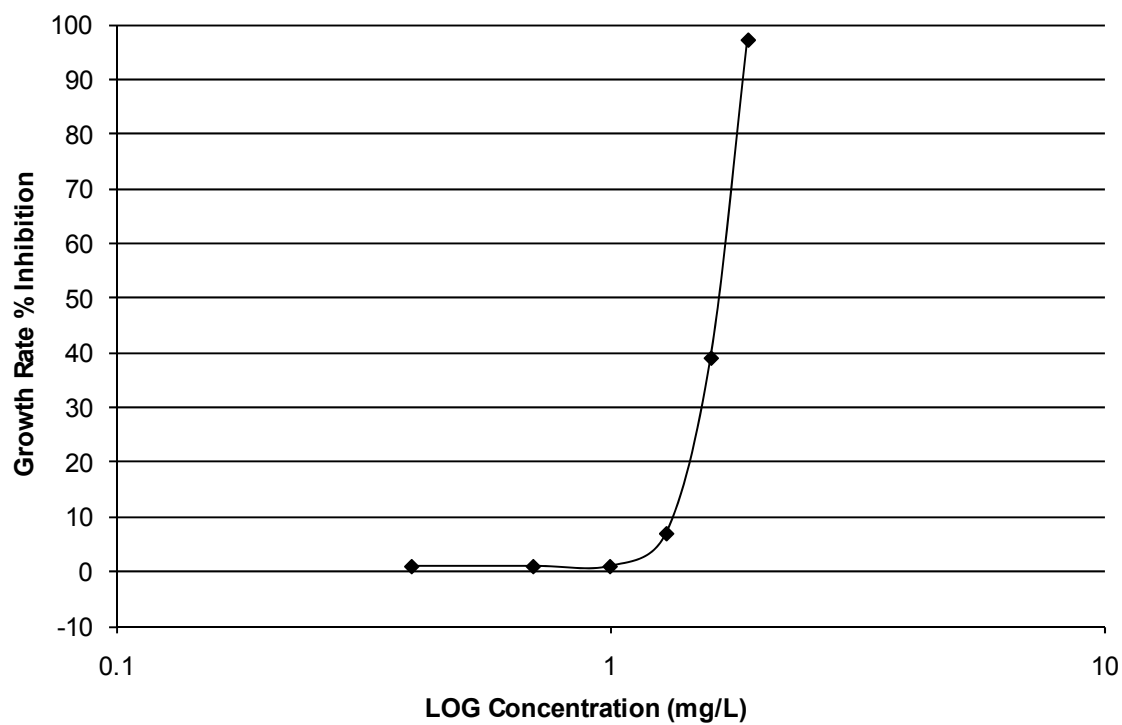
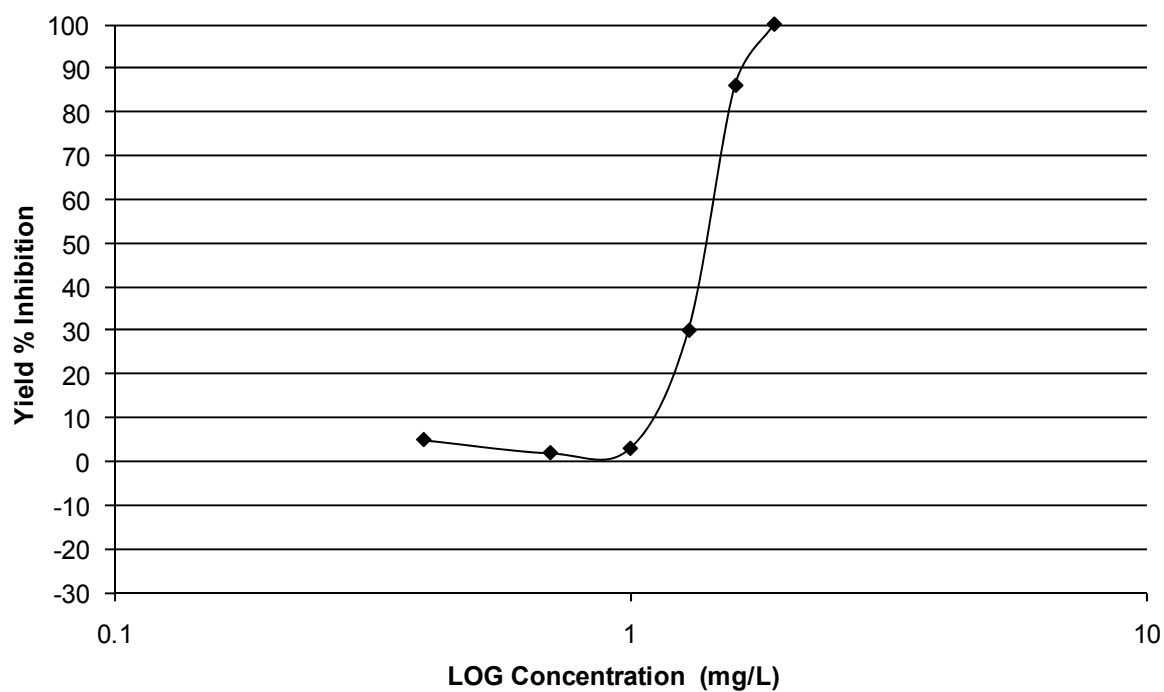


Figure 3. Plot of Percent Inhibition of *Pseudokirchneriella subcapitata* Yield as Compared to the Log Concentration after 96-Hour Exposure to Naphthenic Acids



**APPENDIX A – TEST SUBSTANCE PHYSICAL-CHEMICAL
SPECIFICATIONS FROM SUPPLIER**

REVISION 4: March 28, 2008	MATERIAL SAFETY DATA SHEET	Page 1/6	
1 IDENTIFICATION OF THE PRODUCT AND OF THE COMPANY			
1.1 Identification of the Product:	Naphthenic Acids (Carboxylic Acids, Fatty Acids)		
1.2 Product Code:	NAP ACID		
1.3 Company:			
1.4 Transportation Emergency:	USA	1-800-424-9300 (CHEMTREC)	
1.5 Product Information:	1-205-556-1556 1-205-556-0568 (Fax)		
1.6 Intended Use:	For industrial use only. No other use is intended.		
2 HAZARDS IDENTIFICATION			
2.1 Classification:	Irritant (Xi); Harmful (Xn)		
2.2 Warning Statements:	Causes eye and skin irritation. Harmful if swallowed – may enter lungs if swallowed or vomited. High vapor concentrations may cause drowsiness and irritation of the eyes or respiratory tract.		
2.3 Hazard Symbol(s):			
2.4 Risk Phrases:	R36/38 (Irritating to eyes and skin); R65 (Harmful: May cause lung damage if swallowed)		
2.3 Potential Health Effects:	Eye Contact: Causes eye irritation. Exposure may cause irritation, redness and tearing. Skin Contact: Causes skin irritation. Exposure may cause redness, itching and inflammation. Ingestion: Expected to be a low ingestion hazard. Aspiration hazard. If swallowed, can enter lungs and cause damage. Inhalation: High vapor concentrations may cause drowsiness, respiratory tract irritation, coughing, asthmatic breathing and breathlessness. Chronic Effects: No known deleterious effects.		
2.4 Other Hazards:	Target organs: Eyes, skin and central nervous system.		
3 COMPOSITION/INFORMATION ON INGREDIENTS			
Substance	CAS No.	EC No.	% Present
Naphthenic Acid	1338-24-5	215-662-8	70 - 99
Petroleum Distillates	8008-20-6	232-366-4	1 - 30

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MATERIAL SAFETY DATA SHEET

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Naphthenic Acids (Carboxylic Acids, Fatty Acids)

4 FIRST-AID MEASURES

- In case of contact with eyes: Promptly flush eyes with running water for 15 minutes, including under eyelids. Seek medical assistance if irritation develops.
- In case of contact with skin: Wash affected area well with soap and water. Seek medical assistance if irritation develops.
- In case of ingestion: **Do not induce vomiting.** Give 2-3 glasses of milk or water to dilute. Contact physician or poison control center promptly for instructions. **If vomiting occurs, keep head lower than hips to help prevent aspiration. Never give anything by mouth to an unconscious person.**
- In case of inhalation: Remove to fresh air. Seek medical assistance if irritation develops.

5 FIRE-FIGHTING MEASURES

- 5.1 Suitable extinguishing media: Water fog, fire fighting foam, dry chemical or carbon dioxide.
- 5.2 Unsuitable extinguishing media: None
- 5.3 Specific hazards: Combustion products are Carbon Oxides.
- 5.4 Personal protective equipment: Wear Self Contained Breathing Apparatus and protective clothing appropriate for fire-fighting.
- 5.5 Other precautions: **Non-emergency personnel should be removed from the area immediately. Cool fire-exposed containers with water spray. Prevent water runoff from reaching drains, surface water and ground water.**

6 ACCIDENTAL RELEASE MEASURES

- 6.1 Personal precautions: Avoid unnecessary exposure by wearing personal protective equipment specified in Section 8. **Remove material from eyes, skin and clothing.**
- 6.2 Spill cleanup: **Suction up free liquids using non-sparking equipment. Liquid unable to be suctioned may be absorbed with a non-combustible material (vermiculite, sand, earth, etc.) and transferred to container(s) for later disposal. Remove contaminated sand, earth, etc. and transfer to container(s) for later disposal.**
- 6.3 Environmental precautions: **Keep away from drains, surface water and ground water.**

7 HANDLING AND STORAGE

- 7.1 Handling: Wear appropriate personal protective equipment (see Section 8).
- Avoid breathing vapors, mists or spray. Use with adequate ventilation.
- Avoid contact with eyes, skin and clothing.
- Wash thoroughly after handling.
- Do not taste or swallow.**
- 7.2 Storage: Store in a sealed container in a clean, dry, well-ventilated area away from oxidizers, strong bases, heat and flame.
- Avoid use of copper and brass alloys in storage and transfer equipment and process equipment.

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MATERIAL SAFETY DATA SHEET

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Naphthenic Acids (Carboxylic Acids, Fatty Acids)

8 EXPOSURE CONTROLS/PERSONAL PROTECTION

- 8.1 Engineering controls: Use local exhaust ventilation to control emissions at source.
- 8.2 Eye/face protection: Wear safety glasses with side shields as minimum protection. Wear goggles or faceshield if a risk of splashing exists.
- 8.3 Skin protection: Wear chemical resistant gloves. Heavy PVC, butyl rubber or Viton are recommended.
- 8.4 Respiratory protection: An approved respirator must be worn if engineering controls do not maintain airborne concentrations below established exposure limits or, when limits have not been established, below irritant levels. Respirator selection must be based upon the airborne concentration. Consult a health and safety professional or manufacturer for specific recommendations.
- 8.5 Thermal hazards: None
- 8.6 Occupational Exposure Levels

Chemical Name	Source	Type	Exposure Limits	Notes
Cherosene (Non-Aerosol), Come Vapore totale Dell'Idrocarburo	Italy OEL's	TWA	200 mg/m ³	Skin Total Hydrocarbon Vapor
Kerosene	Poland MAC's	TWA	100 mg/m ³	
	Poland MAC's	STEL	300 mg/m ³	
Kerosine	Russian Federation MAC's	Ceiling	300 mg/m ³	As C
	Russian Federation MAC's	TWA	600 mg/m ³	As C
Kerosene (Non-Aerosol), As Total Hydrocarbon Vapor	ACGIH	TWA	200 mg/m ³	Irritation, CNS, Skin
Kerosene	NIOSH	REL	100 mg/m ³	

9 PHYSICAL AND CHEMICAL PROPERTIES

- 9.1 Appearance: Amber color
- 9.2 Odour: Hydrocarbon
- 9.3 pH: 5.2 (Saturated Solution)
- 9.4 Boiling Pt./range: 268°C (515°F)
- 9.5 Freezing Pt./Range: Not established
- 9.6 Flash point: >149°C (300°F)
- 9.7 Flammability: See 9.6
- 9.8 Autoflammability: See 9.6
- 9.9 Explosive properties: Not applicable
- 9.10 Oxidizing properties: Not an oxidizer
- 9.11 Vapor pressure: 0.005 mm Hg
(37.8°C/100°F)
- 9.12 Relative density (H₂O = 1): 0.960 – 0.982 (15.6°C/60°F)
- 9.13 Apparent density: Not applicable
- 9.14 Vapor density (Air = 1): 6.5
- 9.14 Solubility:
- | | | |
|----------------|---|-----------------------------|
| Water | - | <1% by weight (15.6°C/60°F) |
| Fat (type) | - | Not determined |
| Other solvents | - | Not determined |
- 9.15 Partition coefficient: Log P_{O/W} (Octanol/water) - Not determined
- 9.16 Other data: Not Applicable

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MATERIAL SAFETY DATA SHEET

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Naphthenic Acids (Carboxylic Acids, Fatty Acids)

10 STABILITY AND REACTIVITY

- 10.1 Reactivity: Not reactive under specified conditions of storage, shipment and use.
- 10.2 Stability: Stable under specified conditions of storage, shipment and use.
- 10.3 Conditions to avoid: Heat and ignition sources.
- 10.4 Incompatible materials: Strong oxidizers and strong bases.
- 10.5 Hazardous decomposition products: Carbon Oxides.

11 TOXICOLOGICAL INFORMATION

- 11.1 Acute: Eye and skin irritant. Not established as a respiratory tract irritant. May cause lung damage if aspirated.

Specified Substances

Chemical Name	Test Results
Kerosene	Dermal LD ₅₀ (Rabbit): >2000 mg/kg
	Oral LD ₅₀ (Rat): >5000 mg/kg
	Inhalation LC ₅₀ : >5000 mg/m ³ , 4 H
	Skin (Rabbit): 500 mg (Severe Irritation)
	Skin (Rabbit): 100%/24 H (Moderate Irritation)
Naphthenic Acid	Oral LD ₅₀ (Rat): 3000 mg/kg
	Oral LD ₅₀ (Rat): 5880 mg/kg
	Dermal LD ₅₀ (Rabbit): >3160 mg/kg
	Eye (Rabbit): Moderate
	Skin Occluded (Rabbit): Moderate to Severe
	Skin (Rabbit): Slight

- 11.2 Chronic: Neither ingredient is listed by NTP, IARC or OSHA as a carcinogen. Kerosene (Non-Aerosol), as total hydrocarbon vapor, is listed by ACGIH as A3 (Confirmed Animal Carcinogen).

12 ECOLOGICAL INFORMATION

- 12.1 Ecotoxicity: No data available.
- 12.2 Persistence and degradability: No data available.
- 12.3 Bioaccumulation potential: No data available.
- 12.4 Mobility in soil: No data available.
- 12.5 Other adverse effects: No data available.

13 DISPOSAL CONSIDERATIONS

Generators of waste material are responsible for evaluating materials for compliance with all applicable procedures and regulations. Disposal of unused materials must be in accordance with all local, state and federal regulations. Containers should be cleaned of residual product and rinsed according to all local, state and federal regulations prior to disposal.

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Naphthenic Acids (Carboxylic Acids, Fatty Acids)

14 TRANSPORT INFORMATION

	UN Number	Proper Shipping Name	Hazard Class(es)	Packing Group
ADR/RID:	Not regulated			
IMO/IMDG:	Not regulated	Fatty Acids (Saturated C13+)		
IATA:	Not regulated			
DOT:	NA3082	Other Regulated Substances, Liquid, n.o.s., (Naphthenic Acid)	9	III

Note: Material is regulated by DOT only if shipped in a container containing an amount equal to or greater than the Reportable Quantity (RQ) of 100-pounds.

15 REGULATORY INFORMATION

Warning symbol:



Warning words:

Harmful. Irritant.

Risk phrases:

R36/38: Irritating to eyes and skin

R65: May cause lung damage if swallowed

Safety phrases:

S23: Do not breathe vapor

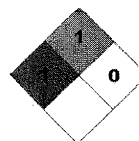
S24/25: Avoid contact with eyes and skin

S62: If swallowed, do not induce vomiting. Seek medical advice immediately and show this container or label

HMIS ratings (estimated):

HEALTH	1
FLAMMABILITY	1
REACTIVITY	0
PHYSICAL HAZARD	

NFPA ratings (estimated):



SARA:

Section 302: None

Section 311/312: Immediate Health Hazard

Section 313: None

WHMIS:

D2B

Inventories:

CAS Number
1338-24-5
8008-20-6

TSCA
Yes
Yes

DSL
Yes
Yes

EINECS
Yes
Yes

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Naphthenic Acids (Carboxylic Acids, Fatty Acids)16 OTHER INFORMATIONRevision Date: March 28, 2008Supersedes Revision Date: November, 2004Revisions: The latest informational changes are indicated by 20% shading.

The information on this form is furnished solely for the purpose of enabling those who transport, handle or use our products to ensure the safety and health of their employees and to comply with various laws and regulations (federal, state and local). This information is offered in good faith and is believed to be accurate. however, makes no guarantee or warranty, expressed or implied, regarding the accuracy of these data or the results to be obtained from the use hereof.

SAMPLE SUMMARY: SAMPLE FOR API/HPV TESTING

Specification	Results	Procedure
Acid number	235mg KOH/gm	ASTM D664-59
Unsaponifiabiles (Total)	4.9%	ASTM D322
Viscosity @ 40	32cst	ASTM D445-88
Specific Gravity @ 20C	0.969	ASTM D1298-85
Color (Gardner), GI	4.5	ASTMD1544-80
Water Content	0.07%	ASTM D95-83
Phenolic Content (acid)	0.31%	Standard Methods for the Examination of Water and Wastewater, 14 th Edition (1975); Method 510, pp 574-592, APHA-AWWA-WPCF
Total Sulfur	0.34	ASTM D4294-83
CP - Flash Point °F (COC)	343	ASTM D92

APPENDIX B – ABC WELL WATER CHARACTERIZATION

Chemical Characteristics of ABC Well Water Used by
ABC Laboratories' Chemical Services Group

August 2009 ABC Well Water Screen (non-GLP)					
Organophosphate (µg/L)	2009	Historical Range 1998-2009 ¹	Elements (mg/L)	2009	Historical Range 1998-2009 ¹
Azinphos ethyl	---	<1.0 ⁵	Aluminum	<0.0500	<0.0500 ⁴
Azinphos, methyl	<0.200	<0.200 ³	Antimony	<0.0500	<0.0500 ⁴
Bolstar	<0.200	<0.200 ⁴	Arsenic	<0.0250	<0.0250-<0.050
Chloropyrifos	<0.200	<0.200 ⁴	Barium	0.0189	0.0189 ⁴
Coumaphos	<0.400	<0.400 ⁴	Beryllium	<0.0010	<0.0010 ⁴
Demeton, Total	<0.200	<0.200-<1.0	Boron	0.400	0.37-0.415
Diazinon	<0.200	<0.200-<1.0	Cadmium	<0.0020	<0.0020-<0.0050
Dichlorvos	<0.200	<0.200 ⁴	Calcium ²	76.3	52-83.1
Dimethoate	<1.00	<1.00 ⁴	Chromium	<0.0100	<0.0100
Disulfoton	<0.200	<0.200-<1.0	Cobalt	<0.0100	<0.0100 ⁴
EPN	<0.200	<0.200 ⁴	Copper	<0.0100	<0.0100
Ethion	---	<1.0 ⁵	Iron	0.020	<0.0059-0.16
Ethoprop	<0.200	<0.200 ⁴	Lead	<0.0400	<0.0065-0.0400
Fensulfothion	<1.00	<1.00 ⁴	Magnesium ²	30.7	27-33.1
Fenthion	<0.200	<0.200 ⁴	Manganese	<0.0050	<0.0050 ⁴
Malathion	<0.200	<0.200-<1.0	Molybdenum	<0.0100	<0.0100 ⁴
Merphos	<0.200	<0.200 ⁴	Mercury	---	<0.00060 ⁵
Mevinphos	<1.00	<1.00 ⁴	Nickel	<0.0100	<0.0100-<0.020
Monocrotophos	<1.00	<1.00 ⁴	Potassium ²	7.51	6.6-7.93
Naled	<2.00	<2.00 ⁴	Selenium	<0.0500	<0.050
Parathion:	---	<1.0 ⁵	Silver	<0.0100	<0.010
Parathion, Ethyl	<0.200	<0.200 ³	Sodium ²	29.0	27-32.2
Parathion, Methyl	<0.200	<0.200 ³	Thallium	<0.0500	<0.0500 ⁴
Phorate	<0.200	<0.200 ⁴	Tin	<0.0200	<0.0200 ⁴
Ronnel	<0.200	<0.200 ⁴	Vanadium	<0.0100	<0.0100 ⁴
Stirophos	<0.200	<0.200 ⁴	Zinc	0.0197	0.0118-0.078
Sulfotepp	<0.200	<0.200 ⁴	Chlorinated		
TEPP	<0.200	<0.200 ⁴	Hydrocarbons (µg/L)		
Tokuthion	<0.200	<0.200 ⁴	4,4'-DDD	<0.04	<0.040
Trichloronate	<0.200	<0.200 ⁴	4,4'-DDE	<0.04	<0.040
			4,4'-DDT	<0.04	<0.040
			Aldrin	<0.04	<0.040
			α-BHC	<0.04	<0.040
			β-BHC	<0.04	<0.040
			Δ-BHC	<0.04	<0.040
			Dieldrin	<0.04	<0.040
			Endosulfan I	<0.04	<0.040
			Endosulfan II	<0.04	<0.040
			Endosulfan sulfate	<0.04	<0.040
			Endrin	<0.04	<0.040
			Endrin aldehyde	<0.04	<0.040
Polychlorinated					
Biphenyls (µg/L)					
Aroclor 1016	<1.00	<1.00			
Aroclor 1221	<1.00	<1.00			
Aroclor 1232	<1.00	<1.00			
Aroclor 1242	<1.00	<1.00			
Aroclor 1248	<1.00	<1.00			
Aroclor 1254	<1.00	<1.00			
Aroclor 1260	<1.00	<1.00			

Chemical Characteristics of ABC Well Water Used by
ABC Laboratories' Chemical Services Group (continued)

August 2009 ABC Well Water Screen (non-GLP)					
<u>Miscellaneous (mg/L)</u>	<u>2009</u>	<u>Historical Range 1998-2009¹</u>	<u>Chlorinated Hydrocarbons (µg/L) (continued)</u>	<u>2009</u>	<u>Historical Range 1998-2009¹</u>
Nitrite N	0.01	0.01-≤0.050	Endrin Ketone	<0.04	<0.040
Nitrate N	0.328	<0.11-0.328	γ-BHC	<0.04	<0.040
Total Phosphorus as P	<0.12	<0.050-0.64	Heptachlor	<0.04	<0.040
Chlorinated Herbicides (µg/L)			Heptachlor epoxide	<0.04	<0.040
2,4,5-TP (silvex)	<0.200	<0.200-<50	Methoxychlor	<0.04	<0.04-<0.095
2,4-D	<0.200	<0.200-<250	Toxaphene	<0.50	<0.50-<3.8
			Chlordane	<0.05	<0.05-<0.48

¹ Data supporting these values are on file at ABC Laboratories. Less than (<) values indicate recovery was below the greatest limit of detection during these analyses.

² Historical Range is from 2003.

³ Historical Range is from 2008.

⁴ Historical Range is from 2009.

⁵ Historical Range does not include 2009.

Note: ABC well water is the base water for ABC Reagent Water.

APPENDIX C – PROTOCOL AND AMENDMENTS

**Growth Inhibition Test of Water Accommodated
Fractions of Naphthenic Acids to the Unicellular
Green Alga, *Pseudokirchneriella subcapitata***

ABC Study No. 64405

This protocol complies with Ecological Effects
Test Guideline OPPTS 850.5400 and OECD Guideline 201

This protocol is based upon ABC generic protocol G101.

1.0 STUDY TITLE

Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

2.0 OBJECTIVE

The objective of this test is to define the concentration response curve and determine the 72-hour and the 96-hour EL₅₀ and EC₅₀ values (i.e., E_rL₅₀, E_yL₅₀, and EL₅₀/E_rC₅₀, E_yC₅₀, and EC₅₀), if possible, of the test substance with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) under static test conditions. Reduction in algal cell density, algal growth rates, and yield in test substance treatments relative to the controls will be used to evaluate the phytotoxicity of the test substance. Any changes in cell morphology will also be noted. In addition, if possible, a no-observed-effect concentration (NOEC) and loading rate (NOELR) at 72- and 96-hours will be determined.

3.0 STUDY SPONSOR

American Petroleum Institute
1220 L Street, NW
Washington, DC 20005
Phone: (202) 682-8480 Fax: (202) 682-8270
Sponsor Representative: [REDACTED]

Study Monitor: [REDACTED]
EcoTox Assessments LLC
506 Tennant Circle, Suite 100
St. Michaels, Maryland 21663
Tel: 410-745-6172 Fax: 410-745-9161
E-mail: [REDACTED]

4.0 TESTING FACILITY AND STUDY DIRECTOR ADDRESS

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Study Director: [REDACTED]
TEL: (573) 777-6385 FAX: (573) 777-6089
Email: [REDACTED]

5.0 PROPOSED SCHEDULE

PROPOSED EXPERIMENTAL START DATE: February 2009

PROPOSED EXPERIMENTAL COMPLETION DATE: March 2009

6.0 TEST PROTOCOL

The test protocol that follows is based on U.S. EPA OPPTS guideline 850.5400 (1) and OECD guideline 201 (2). Modifications to the regulatory guidelines were made to address the testing of insoluble and complex mixtures (3, 4).

7.0 TEST AND REFERENCE SUBSTANCES**7.1 Test Substance**

The test substance will be Naphthenic Acids (CAS# 1338-24-5). The following sample information and chemical/physical properties should be provided with the test substance sample or before its shipment: batch/lot number, sample expiration date, physical description, purity (including certificate of analysis), stability, suggested storage conditions, water and organic solvent solubility, vapor pressure, available toxicity information, a Material Safety Data Sheet (MSDS) or its equivalent, and handling precautions.

7.2 Reference Substance

The reference substance will be Naphthenic Acids (CAS# 1338-24-5). The same information specified for the test substance sample in section 7.1 should be provided for the reference substance sample.

7.3 Sample Characterization and Retention

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

7.4 Test Substance Preparation/Addition

Test solutions will be prepared as water-accommodated fractions (WAF), with each WAF being independently prepared. The WAFs will be prepared by adding the test substance to dilution water on a weight/volume basis and will be reported as the loading rate of test substance per volume of dilution water. The maximum loading of the test substance in a WAF preparation is not to exceed 1,000 mg/L. The WAF preparation will be prepared in an appropriately sized vessel made of glass and will be stirred with magnetic stir bar. WAF vessels will be filled as to maintain minimum head space and covered with a rubber stopper or parafilm. The stirring speed will be adjusted so that the vortex in each bottle does not extend greater than approximately 30-50% of the water column. Stirring will take place at ambient room temperature and lighting. After the prescribed time of stirring, stirring will be stopped and the mixture allowed to sit undisturbed for approximately 1 hour before initiating drawing/siphoning of the WAF solution. Trials of the WAF preparations and equilibration will be performed as part of ABC study 64403 to determine the optimum mixing duration to maximize the soluble fraction of the test substance in dilution water. The containers and preparations used in this study will be described by protocol amendment and summarized in the final report.

8.0 TEST SYSTEM**8.1 Species**

The test species will be the unicellular green alga, *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*. The species will be initially identified by the supplier and then periodically inspected to insure the purity and identity of the culture.

8.2 Justification

When freshwater algal toxicity data are generated following OECD guideline 201 and OPPTS guideline 850.5400, *Pseudokirchneriella subcapitata* is one of the recommended species.

8.3 Source

Pseudokirchneriella subcapitata (UTEX 1648) will be obtained from an established ABC Laboratories' culture which originated with an inoculum received from the University of Texas, Austin, Texas.

8.4 Age

Transfers will be made regularly into fresh algal nutrient medium to provide 3- to 4-day old cultures, which conforms with OECD Guideline 201, for test inoculations.

8.5 Culture

The algae will be cultured in freshwater algal nutrient medium under continuous illumination of approximately 4,300 lux, which conforms with OPPTS guideline 850.5400, at a temperature of $24 \pm 2^{\circ}\text{C}$.

9.0 TEST MEDIUM

The test medium will be filtered (0.45 micrometers) freshwater algal growth medium prepared with ABC reagent water and reagent grade chemicals (5). The pH of the medium prior to inoculation will be 7.5 ± 0.1 and may be adjusted to this pH with 0.1N NaOH or HCl prior to test substance addition. Depending upon the situation, the pH may be adjusted following test substance addition, but prior to inoculation of algae. The base water, i.e., ABC well water, used to prepare the dilution water is chemically characterized as per ABC SOP to verify that it is free of contaminants that might interfere with test results.

10.0 TEST PROCEDURES

Generally two toxicity tests will be conducted, a range-finding and definitive test. The range-finding test is an abbreviated toxicity test employing widely spaced test concentrations to define the approximate range within which the test substance produces a gradient from nontoxic to toxic effects. The range-finding test is conducted using the same basic procedures and conditions as those used during definitive tests. Results of the range-finding test(s) guide selection of the test concentrations for the definitive test, the purpose of which is to provide a precise estimate of the 72- and 96-hour median effective loading rate (EL_{50}) of the test substance which affects the growth of this alga.

10.1 Range-Finding Test

The range-finding test(s) will be initiated by inoculating at least one flask per test substance WAF concentration with a predetermined aliquot of algal inoculum. WAF concentrations will typically cover several orders of magnitude (e.g., nominal loading rates of 1.0, 10, 100, and 1,000 mg/L). Typically, test condition parameters such as light intensity, oscillation rate, test solution pH and temperature will be measured at test initiation and termination. Additional or fewer measurements may be made at

the discretion of the Study Director. At a minimum, cell counts will be determined after approximately 96 hours of exposure.

10.2 Definitive Test

10.2.1 Experimental Design

The definitive test will consist of one or more control treatments and a geometric series (ratio between concentrations ≤ 3.2) of at least five test substance WAF loading rates. Definitive test substance loading rates will be specified by protocol amendment. All test chambers will be labeled with the following information for identification purposes: ABC study number, treatment (e.g., control, vehicle control, level 1, level 2, etc.), replicate (e.g., A, B, etc.), and grid position.

The definitive test will generally be conducted in 250-mL Erlenmeyer flasks fitted with foam stoppers to permit gas exchange and to prevent contamination. If 250-mL flasks are used, each flask will contain 100 mL of test solution. The size of the Erlenmeyer flasks is not critical, but the sample-to-volume ratio should not exceed 50%. Flasks used in testing will be cleaned and sterilized (autoclaved) according to the ABC Laboratories' SOP.

At least three replicates will be used for each test substance loading rate and preferably six replicates for the control. Additional replicates may be prepared for analytical verification of the test substance at 72 hours. Each replicate will be inoculated with algae and placed on a rotary shaker at approximately 100 revolutions per minute. For the control of bias among replicates, test flasks used during the definitive test will be assigned to the testing area using a computer-generated randomization table. The algal inoculum will be from a 3- to 4-day-old stock culture or a culture, growing exponentially, with a sufficient cell density to yield a final inoculum density of at least 1×10^6 cells/mL. The test algae will be inoculated into the test flasks within 30 minutes after preparation of test solutions to yield an approximate initial cell density of at least 1×10^4 cells/mL. This route of administration was chosen in order to comply with OECD 201 and OPPTS 850.5400 testing guidelines. The test flasks will then be incubated in a temperature-controlled enclosure illuminated continuously for 96 hours.

A limit test may be performed at a nominal loading rate of 1,000 mg/L or up to the limit of solubility of the substance in the medium (whichever is lower)

for situations in which the algal growth inhibition is estimated to be less than 50% at this maximum test concentration. The limit test concentration will be replicated six times, with the same number of controls. If during the limit test, a mean decrease of 25% or more is found in either growth rate or yield between the limit test and the control, a full multi-concentration definitive test should be carried out.

10.2.2 Lighting and Oscillation

Throughout the test, the test flasks will be illuminated continuously by cool-white fluorescent bulbs that provide $4,300 \pm 430$ lux light intensity, as recommended by OPPTS 850.5400 guideline for the 96-hour exposure, and continuously swirled on an orbital shaker table at approximately 100 rpm. Daily light reading (measured at the level of the test solutions) and shaker oscillation rate will be measured and recorded.

10.2.3 Chemical/Physical Parameters

Temperature of the environmental chamber will be measured continuously during the definitive test. Temperature and pH will be measured at 0 and 96 hours in all control and test substance treatments. Temperature and pH measurements will also be made at 72 hours in an additional replicate set up exclusively for water quality and analytical measurements at 72 hours. Measurements at test initiation (0-hour) will be conducted on all parent solutions (prior to distribution of the solutions to the test flasks). Measurements at 96 hours will be conducted on at least one replicate of each control and test substance treatment. The temperature will be maintained at $24 \pm 2^{\circ}\text{C}$. Additional water quality measurements may be made at the discretion of the Study Director.

10.2.4 Biological Data

Cell density will be determined for each replicate of the control and each test loading rate at 24, 48, 72, and 96 hours (± 1 hour from test initiation) to evaluate algal growth (inhibition or enhancement). Cell density may also be determined at 0 hour for each replicate of the control to confirm initial cell densities. Cell density determinations will be accomplished using a hemacytometer and an optical microscope. In addition to cell density determinations, microscopic examination will be conducted to determine if there are any morphological or physical effects on the algal cells. Unusual

cell shapes, color differences, flocculation, adherence of algae to test chambers, or aggregation of algal cells will be noted.

In treatments where growth is maximally inhibited (i.e., cell numbers in treatment are less than or equal to the initial cell density), algistatic effects will be differentiated from algicidal effects. This will be accomplished by removing 0.5-mL aliquots of test solution containing growth-inhibited algae from each replicate test flask. The aliquots will be combined for each affected test concentration in a new test flask and diluted with fresh nutrient medium to a test concentration that does not affect growth. A control will be set up at an initial cell density of 10,000 cells/mL to confirm the acceptability of the procedures. The subculture(s) will be incubated under the environmental conditions of the definitive test for a maximum of 9 days in order to make observations for algal growth. The subculture will be discontinued as soon as growth occurs.

10.2.5 Analytical Confirmation

The concentrations of the total dissolved naphthenic acids in the WAFs will be measured in all control and test substance treatments at 0 and 96 hours of the range-finding test. The concentration of the test substance will be measured in all control and test substance treatments at 0, 72, and 96 hours of the definitive test. Time 0-hour samples will be collected from parent solutions. Time 72-hour samples will be collected from additional replicates prepared for analytical verification at this time point after algal cells are removed by centrifugation or filtration, as appropriate. Time 96-hour samples will be collected after combining replicate solutions by treatment and algal cells are removed by centrifugation or filtration, as appropriate. A minimum of two fortification spikes (quality control samples) will be prepared and analyzed with each sample set. An extra replicate containing the lowest test substance treatment without algae will be included and analyzed at test termination to determine if the test substance may have been incorporated into the algal biomass during the period of the test. Additional flasks per treatment may be prepared if necessary for analytical confirmation purposes.

The analysis of the samples for the test substance will be based on an analytical method provided by the Sponsor and validated at ABC Laboratories. The analytical method will be described by protocol amendment to this protocol after validation.

11.0 ANALYSIS OF RESULTS

The results of the definitive test will be examined to determine those concentrations that inhibit or enhance growth of the test algae. These values will be determined by the following equation:

$$\left(\frac{\text{Control Cell Density} - \text{Treatment Cell Density}}{\text{Control Cell Density}} \right) \times 100 = \% \text{ Inhibition}$$

Results will be reported using the nominal WAF loading rates and measured initial concentration of total dissolved naphthenic acids when measured values are within $\pm 20\%$ of the nominal or measured initial concentration. If the deviation from the nominal or measured initial concentration is greater than $\pm 20\%$, results will be based on the nominal WAF loading rates and the geometric mean concentration during the exposure. When requested by the Sponsor, alternate methods of reporting the results may be used in conjunction with the above methods.

The results of the definitive test will be used to calculate the 72- and 96-hour median effect loading rate and concentration values for specific growth rates ($E_r L_{50}/E_r C_{50}$) and yield ($E_y C_{50}/E_y C_{50}$) and their 95% confidence limits. Other EC_x or EL_x values (e.g., EL/EC_{10} , EL/EC_{20} , etc.) and their 95% confidence limits may be estimated, if possible, for these endpoints or for other endpoints (e.g., cell density, etc.) at the discretion of the Study Director or as directed by the Sponsor. A goodness-of-fit determination will be determined for the concentration-response curve. These values may be determined by the SAS nonlinear modeling procedure (four parameter logistic model with two parameters fixed) or a different curve fitting application. The method used to generate the concentration-response curves will be identified in the final report. In addition, the no-observed-effect concentration (NOEC) and loading rate (NOELR) for at least 72 and 96 hours will be determined by using a one-way analysis of variance (ANOVA) and a multiple means comparison test using the individual replicate values for the specific growth rate or yield. Additional effect concentrations may be estimated for other time points (e.g., 24- or 48-hours) for these endpoints or for other endpoints (e.g., cell density, etc.) at the discretion of the Study Director.

12.0 TEST ACCEPTABILITY CRITERIA

The number of algal cells in the control(s) after the initial 72 hours of the test should be at least 16 times the initial inoculum density and the final density should be approximately 1×10^6 cells/mL after 96 hours of testing to verify logarithmic phase growth during the test duration. The coefficient of variation for daily growth rates in the control replicates during the course of the test must not exceed 35% during the first 72 hours of testing to satisfy the

OECD requirements. The coefficient of variation of average specific growth rates during the initial 72 hours in control replicates must not exceed 7%, also to satisfy the OECD requirements. In addition, the coefficient of variation for cell numbers in the control replicates at 96 hours should not vary by more than 20% to satisfy the OPPTS requirements. Unless the maximum loading rate of 1000 mg/L is tested, one test concentration should exhibit $\leq 50\%$ decrease in growth and one concentration should exhibit $\geq 50\%$ decrease in growth relative to the control(s). The pH in the control should not increase more than 1.5 units after the initial 72 hours to satisfy the OECD acceptability requirements for pH.

13.0 REPORT

Upon completion of the range-finding test, a summary report will be submitted to the Sponsor. The summary report will briefly describe the test methods and test results. A Final report detailing all aspects of the study will be submitted to the Sponsor and will include, but not be limited to, the following:

- Study dates, name, and address of test facility.
- Objectives and test procedures as stated in approved protocol.
- A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
- Description of test substance (e.g., date of receipt, storage conditions, method of preparing stock and/or test solution and, if available, purity, physical characteristics, water and organic solvent solubility) and identification of the reference substance, if applicable.
- Description of test conditions during the study (e.g., dilution water, test temperature, lighting, and pH).
- Description of methods used during the study.
- Description of test system (e.g., source, culture techniques, etc.).
- Summary of the data and a statement of the conclusions drawn from any data analyses, if appropriate.
- Description of any protocol deviations.
- Location of raw data.

- List of all study personnel.
- GLP compliance statement by the Study Director and a statement by ABC Laboratories' Quality Assurance Unit.

14.0 PROTOCOL AMENDMENTS AND DEVIATIONS

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

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

15.0 QUALITY ASSURANCE

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

16.0 GLP COMPLIANCE

All test procedures, documentation, records, and reports will comply with the U.S. Environmental Protection Agency's Good Laboratory Practices as promulgated under the Toxic Substances Control Act (6); and OECD Principles of Good Laboratory Practice (7). The report will contain a statement attesting to that fact.

17.0 RECORDS

Records to be maintained will include, but not be limited to, test substance receipt; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until

finalization of the study. Upon completion of the study, all original raw data will be submitted to the Sponsor along with the final report. A copy of the final report, copies of all raw data from the study, and all original facility records will be kept on file in ABC Laboratories' archives.

18.0 SPECIMEN DISPOSAL

Following finalization of the report, disposition of all specimens (i.e., any material derived from the test system for examination, analysis, or retention) generated during the conduct of the test will be completed in a timely manner. Retention specimens holding time will be based on stability information provided by the Sponsor or by stability data generated by ABC Laboratories. Retention specimens will be returned to the Sponsor unless archiving is contracted with ABC laboratories. Documentation of specimen disposal will be retained with study records in ABC Laboratories' Archive.

19.0 REFERENCES

- (1) U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, OPPTS 850.5400, Algal Toxicity, Tiers I and II, EPA712-C-96-164, 9 pp.
- (2) Organization for Economic Cooperation and Development (OECD). March 23, 2006. OECD Guidelines for Testing of Chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guideline No. 201.
- (3) Organization for Economic Cooperation and Development (OECD). 2000. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Series on Testing and Assessment, No. 23. ENV/JM/MONO(2000)6, OECD, Paris, France.
- (4) Girling, A.E., F.G. Whale, and D.M.M. Adema. 1994. A Guideline Supplement for Determining the Aquatic Toxicity of Poorly Water-Soluble Complex Mixtures Using Water-Accommodated Fractions. *Chemosphere* 29(12):2645-2649.
- (5) American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM Designation E1218-97a, 14 pp.
- (6) U.S. Environmental Protection Agency. Toxic Substances Control Act; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*, 54(158): 34043-34050
- (7) Organization for Economic Cooperation and Development. 1997. Decision of the Council, Revised Principles of GLP [C(97)186/Final].

PROTOCOL APPROVAL

Study Director

Name (signed): [REDACTED] Date: 24 FEB 09

Name/Title: [REDACTED]

Sponsor Representative

Name (signed): [REDACTED] Date: 23 Feb 2009

Name/Title: [REDACTED]

Test Facility Management

Name (signed): [REDACTED] Date: 24 Feb 09

Name/Title: [REDACTED]

QAU Protocol Review for GLP Compliance

Name (signed): [REDACTED] Date: 26 Feb 09

Name/Title: [REDACTED]

PROTOCOL AMENDMENT NOTIFICATION

PROTOCOL TITLE:	Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>		
TEST FACILITY:	ABC Laboratories, Inc.	ABC STUDY NO.:	64405
STUDY SPONSOR:	American Petroleum Institute		
AMENDMENT NO.:	1	EFFECTIVE DATE:	Oct. 5, 2009

1. Protocol Section: 7.4 – Test Substance Preparation/Addition

Test solutions will be prepared as water-accommodated fractions (WAF), with each WAF being independently prepared. The WAFs will be prepared by adding the test substance to dilution medium on a weight/volume basis and will be reported as the loading rate of test substance per volume of dilution water. The maximum loading of the test substance in a WAF preparation will not to exceed 1,000 mg/L. The WAF preparation will be prepared in an appropriately sized vessel made of glass and will be stirred with magnetic stir bar. WAF vessels will be covered with a screw cap. The stirring speed will be adjusted so that the vortex in each bottle does not extend greater than approximately 30-50% of the water column. Stirring will take place at ambient room temperature and lighting. After the prescribed time of stirring, stirring will be stopped and the mixture allowed to sit undisturbed for approximately 1 hour before initiating drawing/siphoning of the WAF solution.

WAF preparations during the range-finding test will be prepared at a volume of 2L in clean 2-L glass aspirator bottles, each containing a 2 inch Teflon-coated stir bar. Each WAF will be stirred for 24 hours $\pm$ 1 hour before being allowed to sit undisturbed and settle for approximately 1 hour before collection. WAFs prepared for the range finding test will be collected by draining the solutions from the outlet of each aspirator bottle, after the first approximately 100 mL of prepared solution is discarded as waste.

WAF preparations during the definitive test will be prepared at a volume of 4 L in clean 4-L glass carboys, each containing a 2 inch Teflon-coated stir bar. Each WAF will be stirred for 24 hours $\pm$ 1 hour before being allowed to sit undisturbed and settle for approximately 1 hour before collection. WAFs prepared for the definitive test will be collected by siphoning the prepared solution from each carboy with a glass tube into a clean collection vessel. The first approximately 100 mL of prepared solution from each WAF will be discarded as waste to avoid the collection of any insoluble test substance.

Reason: Describe the containers and preparations used in range finding and definitive tests.

Effect on Study Integrity: None.

2. Protocol Section: 10.2.1 – Experimental Design

The definitive test will be performed at the target nominal loading rates of 0 (control), 2.5, 5.0, 10, 20, 40, and 80 mg Naphthenic Acids/L. All test chambers will be labeled with the following information for identification purposes: ABC study number, treatment (e.g., control, vehicle control, level 1, level 2, etc.), replicate (e.g., A, B, etc.), and grid position.

The definitive test will generally be conducted in 250-mL Erlenmeyer flasks fitted with foam stoppers to permit gas exchange and to prevent contamination. If 250-mL flasks are used, each flask will contain 100 mL of test solution. The size of the Erlenmeyer flasks is not critical, but the sample-to-volume ratio should not exceed 50%. Flasks used in testing will be cleaned and sterilized (autoclaved) according to the ABC Laboratories' SOP.

At least three replicates will be used for each test substance loading rate and preferably six replicates for the control. Additional replicates may be prepared for analytical verification of the test substance at 72 hours. Each replicate will be inoculated with algae and placed on a rotary shaker at approximately 100 revolutions per minute. For the control of bias among replicates, test flasks used during the definitive test will be assigned to the testing area using a computer-generated randomization table. The algal inoculum will be from a 3- to 4-day-old stock culture or a culture, growing exponentially, with a sufficient cell density to yield a final inoculum density of at least 1×10^6 cells/mL. The test algae will be inoculated into the test flasks within 30 minutes after preparation of test solutions to yield an approximate initial cell density of at least 1×10^4 cells/mL. This route of administration was chosen in order to comply with OECD 201 and OPPTS 850.5400 testing guidelines. The test flasks will then be incubated in a temperature-controlled enclosure illuminated continuously for 96 hours.

Reason: Identify the nominal concentrations selected.

Effect on Study Integrity: None.

3. Protocol Section: 10.2.5 – Analytical Confirmation

The concentrations of the total dissolved naphthenic acids in the WAFs will be measured in all control and test substance treatments at 0 and 96 hours of the range-finding test. The concentration of the test substance will be measured in all control and test substance treatments at 0, 72, and 96 hours of the definitive test. Time 0-hour samples will be collected from parent solutions. Time 72-hour samples will be collected from additional replicates prepared for analytical verification at this time point. Time 96-hour samples will be collected after combining replicate solutions by treatment. A minimum of two fortification spikes (quality control samples) will be prepared and analyzed with each sample set. Additional flasks per treatment may be prepared if necessary for analytical confirmation purposes.

A 500 mL sample will be collected and transferred to a 1,000-mL separatory funnel. Each sample will be acidified with concentrated sulfuric acid to a pH level of 2.5 ± 0.1 . A 100-mL

volume of methylene chloride will be added to each sample and the samples shaken to mix. After approximately one minute of shaking, the sample phases will be allowed to separate and the methylene chloride (lower layer) filtered through anhydrous sodium sulfate and collected in a 500-mL flat-bottomed flask. The methylene chloride extraction will be repeated once for each sample. The samples will then be evaporated to dryness using a rotary evaporator and quantitatively transferred to 15-mL culture tubes using two separate 5-mL aliquots of methylene chloride. Each sample will then be evaporated to dryness under a gentle stream of nitrogen and reconstituted with 4 mL of methylene chloride. Each 4 mL sample will be diluted using methylene chloride, if necessary, to produce an analyte concentration that is within the range of the standard curve. The samples will be vialled and analyzed by FT-IR. QC fortifications will be prepared in a similar manner after dilution water had been fortified with naphthenic acids. Sample analysis was performed using a FT-IR system equipped with the following analytical parameters:

Manufacturer: Thermo Nicolet
Model: Avatar 360
Software: Omnic 32
IR Cell: Thermo Scientific, KBr 1.0 mm sealed cell
Cell Holder: Thermo Scientific
Dry Nitrogen Gas Used to Protect the IR Cell Between Runs: Yes
Scan Times: 64
Scan Range: 4000-400 cm^{-1}
Scan Model: Absorbance
Resolution: 4 cm^{-1}
Wave Number of Interest: 1743 cm^{-1}
Solvent Used for Background Collection: Methylene chloride

Note: These instrument parameters may be changed or modified to optimize conditions or to suit the instrument used.

Reason: To provide analytical methodology detail to the protocol.

Effect on Study Integrity: None.

STUDY DIRECTOR:	[REDACTED]	DATE: 090409
TEST FACILITY MANAGEMENT:	[REDACTED]	DATE: 090509

PROTOCOL AMENDMENT NOTIFICATION

PROTOCOL TITLE:	Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>		
TEST FACILITY:	ABC Laboratories, Inc.	ABC STUDY NO.:	64405
STUDY SPONSOR:	American Petroleum Institute		
AMENDMENT NO.:	2	EFFECTIVE DATE:	Jan 18, 2010

1. Protocol Section: 13.0 – Report

A data summary of the range-finding test will be provided to the Sponsor and the results of the range-finding test will be summarized in the final report. A Final report detailing all aspects of the study will be submitted to the Sponsor and will include, but not be limited to, the following:

- Study dates, name, and address of test facility.
- Objectives and test procedures as stated in approved protocol.
- A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
- Description of test substance (e.g., date of receipt, storage conditions, method of preparing stock and/or test solution and, if available, purity, physical characteristics, water and organic solvent solubility) and identification of the reference substance, if applicable.
- Description of test conditions during the study (e.g., dilution water, test temperature, lighting, and pH).
- Description of methods used during the study.
- Description of test system (e.g., source, culture techniques, etc.).
- Summary of the data and a statement of the conclusions drawn from any data analyses, if appropriate.
- Description of any protocol deviations.
- Location of raw data.

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- List of all study personnel.
- GLP compliance statement by the Study Director and a statement by ABC Laboratories' Quality Assurance Unit.

Reason: To clarify how the range-finding results will be reported.

Effect on Study Integrity: None.

STUDY DIRECTOR:	[REDACTED]	DATE: 19 JAN 2010
TEST FACILITY MANAGEMENT:	[REDACTED]	DATE: 19 Jan 10

Amendment No. 2 for ABC Study 64405, Page 2 of 2

PROTOCOL AMENDMENT NOTIFICATION

PROTOCOL TITLE:	Growth Inhibition Test of Water Accommodated Fractions of Naphthenic Acids to the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>		
TEST FACILITY:	ABC Laboratories, Inc.	ABC STUDY NO.:	64405
STUDY SPONSOR:	American Petroleum Institute		
AMENDMENT NO.:	3	EFFECTIVE DATE:	Sept. 24, 2009

1. Protocol Section: 4.0 – Testing Facility and Study Director Address

Additional analytical chemistry identification work will be performed at:
 Department of Biological Sciences
 Z-207 Biological Sciences Centre
 116th Street and 85th Avenue
 University of Alberta
 Edmonton, Alberta T6G 2R3 Canada

Reason: To identify the location where additional analytical work will be performed.

Effect on Study Integrity: None. This is additional work being contracted by the sponsor.

2. Protocol Section: 10.2.5 – Analytical Confirmation

Analytical samples will also be collected at study initiation and sent to Dr. Fedorak at the University of Alberta for analysis.

Reason: To describe the additional analytical samples to be collected for analysis by Dr. Fedorak.

Effect on Study Integrity: None. This is additional work being contracted by the sponsor.

3. Protocol Section: 11.0 – Analysis of Results

Results will be reported using the nominal WAF loading rates and mean measured concentrations of total dissolved naphthenic acids.

Reason: To modify how biological results will be reported.

Effect on Study Integrity: None. Results based on mean measured concentrations requested by the Sponsor for consistency.

4. Protocol Section: 13.0 – Report

The report from Dr. Fedorak's analysis will be presented in an appendix to the final report. Because ABC is not the sponsor of this additional work, ABC is not responsible for its GLP compliance or noncompliance and the corresponding data. A statement regarding these analyses will be added to the Statement of GLP Compliance page in the final report.

Reason: To describe how Dr. Fedorak's analyses will be reported.

Effect on Study Integrity: None. The analyses performed by Dr. Fedorak were additional work contracted by the sponsor.

STUDY DIRECTOR:	[REDACTED]	DATE: 20 JUL 10
TEST FACILITY MANAGEMENT:	[REDACTED]	DATE: 21 JUL 10

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APPENDIX D – REPLICATE CELL DENSITIES

Table D-1. Daily Cell Densities of the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, During a 96-Hour Exposure to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	REP	Cell Density (cells/mL x 10 ⁴)			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	A	3.1	12	39	139
	B	3.3	13	38	138
	C	3.0	13	52	175
	D	3.2	14	55	157
	E	3.3	13	46	155
	F	3.6	14	53	162
2.5	A	3.2	13	45	139
	B	3.2	12	44	146
	C	3.6	12	51	155
5.0	A	2.7	12	40	141
	B	3.6	15	45	158
	C	3.6	12	50	153
10	A	3.9	12	41	165
	B	2.8	10	32	133
	C	2.7	13	47	150
20	A	2.9	6.4	32	125
	B	2.6	7.6	32	104
	C	3.1	8.4	27	94
40	A	1.7	3.3	7.7	20
	B	1.4	3.6	9.7	20
	C	1.6	3.3	8.8	25
80	A	1.1	1.6	1.0	1.0
	B	1.1	1.4	0.89	1.0
	C	1.3	1.3	1.2	1.6

APPENDIX E – REPLICATE GROWTH RATE FROM TIME ZERO

Table E-1. Replicate Growth Rates During a 96-Hour Exposure of *Pseudokirchneriella subcapitata* to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	REP	Growth Rate (cells/mL/hour) ^a			
		0-24 Hours	0-48 Hours	0-72 Hours	0-96 Hours
Control	A	0.0471	0.0518	0.0509	0.0514
	B	0.0498	0.0534	0.0505	0.0513
	C	0.0458	0.0534	0.0549	0.0538
	D	0.0485	0.0550	0.0557	0.0527
	E	0.0497	0.0534	0.0532	0.0525
	F	0.0534	0.0550	0.0551	0.0530
2.5	A	0.0485	0.0534	0.0529	0.0514
	B	0.0485	0.0518	0.0526	0.0519
	C	0.0534	0.0518	0.0546	0.0525
5.0	A	0.0414	0.0518	0.0512	0.0516
	B	0.0534	0.0564	0.0529	0.0527
	C	0.0534	0.0518	0.0543	0.0524
10	A	0.0567	0.0518	0.0516	0.0532
	B	0.0429	0.0480	0.0481	0.0509
	C	0.0414	0.0534	0.0535	0.0522
20	A	0.0444	0.0387	0.0481	0.0503
	B	0.0398	0.0423	0.0481	0.0484
	C	0.0471	0.0443	0.0458	0.0473
40	A	0.0221	0.0249	0.0284	0.0312
	B	0.0140	0.0267	0.0316	0.0312
	C	0.0196	0.0249	0.0302	0.0335
80	A	0.00397	0.00979	0.00	0.00
	B	0.00397	0.00701	-0.00162	0.00
	C	0.0109	0.00547	0.00253	0.00490

^a Values rounded to three significant figures, when available.

**APPENDIX F – SPECIFIC GROWTH RATES FOR CONTROLS AT ADJACENT TIME
POINTS**

Table F-1. Section-by-Section Specific Growth Rates for Controls During a 72-Hour Exposure of *Pseudokirchneriella subcapitata* to Naphthenic Acids

	REP	Section-by-Section Specific Growth Rate (cells/mL/hour) ^a			% Coefficient of Variance	
		0-24 Hours	24-48 Hours	48-72 Hours	Replicate ^b	Overall ^c
Control	A	0.0471	0.0564	0.0491	10	10
	B	0.0497	0.0571	0.0447	12	
	C	0.0458	0.0611	0.0578	15	
	D	0.0485	0.0615	0.0570	12	
	E	0.0497	0.0571	0.0527	7	
	F	0.0534	0.0566	0.0555	3	

^a Values rounded to three significant figures. The section-by-section specific growth rate in each treatment was calculated for each period, i.e., 0 to 24, 24 to 48, and 48 to 72 hours using the following equation:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

where:

μ = average specific growth rate

N_n = cell density at second time point

N_1 = cell density at time point immediately prior to second time point

t_n = second time point

t_1 = time point immediately prior to second time point

^b Replicate % Coefficient of Variance (CV) determined by the following equation:

$$\frac{\text{Standard Deviation of 0-24 hour, 24-48 hour, and 48-72 hour growth rates}}{\text{Mean of 0-24 hour, 24-48 hour, and 48-72 hour growth rates}} \times 100$$

^c Overall % Coefficient of Variance was determined by the following equation:

$$\frac{\text{Rep A CV} + \text{Rep B CV} + \text{Rep C CV} + \text{Rep D CV} + \text{Rep E CV} + \text{Rep F CV}}{\text{Number of Replicates, i.e., 6}}$$

Table F-2. Section-by-Section Specific Growth Rates for Controls During a 96-Hour Exposure of *Pseudokirchneriella subcapitata* to Naphthenic Acids

	REP	Section-by-Section Specific Growth Rate (cells/mL/hour) ^a				% Coefficient of Variance	
		0-24 Hours	24-48 Hours	48-72 Hours	72-96 Hours	Replicate ^b	Overall ^c
Control	A	0.0471	0.0564	0.0491	0.0530	8	10
	B	0.0497	0.0571	0.0447	0.0537	10	
	C	0.0458	0.0611	0.0578	0.0506	13	
	D	0.0485	0.0615	0.0570	0.0437	15	
	E	0.0497	0.0571	0.0527	0.0506	6	
	F	0.0534	0.0566	0.0555	0.0466	8	

^a Values rounded to three significant figures. The section-by-section specific growth rate in each treatment was calculated for each period, i.e., 0 to 24, 24 to 48, 48 to 72, and 72 to 96 hours using the following equation:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

where:

μ = average specific growth rate

N_n = cell density at second time point

N_1 = cell density at time point immediately prior to second time point

t_n = second time point

t_1 = time point immediately prior to second time point

^b Replicate % Coefficient of Variance (CV) determined by the following equation:

$$\frac{\text{Standard Deviation of 0-24 hour, 24-48 hour, 48-72 hour, and 72-96 hour growth rates}}{\text{Mean of 0-24 hour, 24-48 hour, 48-72 hour, and 72-96 hour growth rates}} \times 100$$

^c Overall % Coefficient of Variance was determined by the following equation:

$$\frac{\text{Rep A CV} + \text{Rep B CV} + \text{Rep C CV} + \text{Rep D CV} + \text{Rep E CV} + \text{Rep F CV}}{\text{Number of Replicates, i.e., 6}}$$

APPENDIX G – REPLICATE YIELD

Table G-1. Daily Yield Values of the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, During a 96-Hour Exposure to Naphthenic Acids

Nominal WAF Loading Rate (mg naphthenic acids/L)	REP	Yield (cells/mL $\times 10^4$)			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	A	2.10	11.0	38.0	138
	B	2.30	12.0	37.0	137
	C	2.00	12.0	51.0	174
	D	2.20	13.0	54.0	156
	E	2.30	12.0	45.0	154
	F	2.60	13.0	52.0	161
2.5	A	2.20	12.0	44.0	138
	B	2.20	11.0	43.0	145
	C	2.60	11.0	50.0	154
5.0	A	1.70	11.0	39.0	140
	B	2.60	14.0	44.0	157
	C	2.60	11.0	49.0	152
10	A	2.90	11.0	40.0	164
	B	1.80	9.00	31.0	132
	C	1.70	12.0	46.0	149
20	A	1.90	5.40	31.0	124
	B	1.60	6.60	31.0	103
	C	2.10	7.40	26.0	93.0
40	A	0.700	2.30	6.70	19.0
	B	0.400	2.60	8.70	19.0
	C	0.600	2.30	7.80	24.0
80	A	0.100	0.600	0.000	0.000
	B	0.100	0.400	-0.110	0.000
	C	0.300	0.300	0.200	0.600

**APPENDIX H – CHARACTERIZATION OF NAPHTHENIC ACIDS IN WAF
SOLUTIONS BY GC/MS**

1.0 Toxicity test with algae

1.1 Samples and Methods

In October 2009, two shipments of samples containing the dissolved aqueous fraction (WAFs) of naphthenic acids were received in the Department of Biological Sciences at the University of Alberta. The first shipment contained six samples of aged-blended freshwater from the *Daphnia magna* test. The second shipment contained seven samples of freshwater algal nutrient medium. These were from the Algae test. The concentrations of these were labeled as control (0 mg/L), 2.5, 5, 10, 20, 40, and 80 mg/L. In this report, these samples are referred to as "Algae waters".

An appropriate subsample volume (10 to 200 mL) from each bottle was diluted to 1 L with distilled water and these were extracted individually as outlined by Merlin et al. (2007). Briefly, the diluted sample was acidified to pH 2 with concentrated HCl, and then 150 g NaCl was dissolved into the sample. The water sample was then extracted with three 60-mL portions of DCM. The combined DCM extracts were dried under nitrogen to remove the solvent.

The residue was dissolved in 50 μ L of DCM and the naphthenic acids were derivatized by adding 50 μ L of Sigma MTBSTFA derivatizing agent (without 1% *t*-BDMCS) to each vial and heating at 60°C for 20 min. The derivatized samples were analyzed by GC-MS (Young et al. 2008) and the total ion current mass spectra were collected. The data obtained were put into a Microsoft Excel spreadsheet (Holowenko et al. 2002) to prepare a table of the relative abundances of each ion corresponding to the general formula for naphthenic acids, $C_nH_{2n+Z}O_2$, where *n* is the carbon number and *Z* is zero or a negative even number defining the hydrogen deficiency due to cyclization. The distributions of ions summarized in each table was used to prepare a three-dimensional plots of the ion abundances for each *n* and *Z* value.

1.2 Results and Discussion

The tables of relative abundances of each ion (expressed as percentages) in the seven extracted Algae water samples are shown in Tables 1.1 to 1.7. The values of the percentages reported in these tables were rounded to the nearest 0.1.

Table 1.1

Algae water 0 mg/L

C number	z number							
	0	2	4	6	8	10	12	% carbon no
5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.6
6	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.3
7	1.0	1.3	0.0	0.0	0.0	0.0	0.0	2.3
8	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.6
9	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.7
10	0.2	0.7	0.0	0.0	0.0	0.0	0.0	1.0
11	0.3	0.5	0.1	0.0	0.0	0.0	0.0	0.9
12	3.8	0.5	2.2	0.1	0.0	0.0	0.0	6.6
13	0.7	0.6	0.5	0.0	0.0	0.0	0.0	1.8
14	4.2	0.7	4.6	0.2	0.1	0.0	0.0	9.8
15	1.7	0.1	0.0	0.0	0.0	0.0	0.0	1.9
16	32.1	0.9	0.1	0.0	0.0	0.0	0.0	33.2
17	3.5	0.2	0.0	0.0	0.0	0.0	0.0	3.8
18	22.8	7.9	1.6	0.0	0.0	0.3	0.2	32.8
19	0.7	0.0	0.0	0.0	0.0	0.1	1.5	2.4
20	0.2	0.0	0.0	0.0	0.0	0.2	0.2	0.7
21	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.4
22	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.4
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	72.7	14.8	9.2	0.4	0.1	0.9	2.0	100.0

Table 1.2

Algae water 2.5 mg/L

C number	z number							
	0	2	4	6	8	10	12	% carbon no
5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	1.5
6	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5
7	1.2	1.0	0.0	0.0	0.0	0.0	0.0	2.2
8	0.7	0.4	0.0	0.0	0.0	0.0	0.0	1.1
9	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.5
10	0.2	0.5	0.5	0.0	0.0	0.0	0.0	1.2
11	0.4	3.3	3.3	0.0	0.0	0.0	0.0	7.0
12	1.6	7.7	9.5	0.8	0.0	0.0	0.0	19.6
13	1.9	9.4	10.8	1.8	0.0	0.0	0.0	23.9
14	1.9	6.9	9.5	2.0	0.4	0.0	0.0	20.8
15	1.1	3.4	4.0	1.3	0.2	0.0	0.0	10.0
16	2.4	1.4	1.9	0.7	0.1	0.1	0.0	6.6
17	0.5	0.4	0.6	0.3	0.1	0.0	0.0	1.9
18	1.1	0.9	0.3	0.1	0.0	0.2	0.0	2.6
19	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.4
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	15.2	35.7	40.5	7.1	1.0	0.3	0.2	100.0

Table 1.3

Algae water 5 mg/L

C number	z number							
	0	2	4	6	8	10	12	% carbon no
5	1.4	0.0	0.0	0.0	0.0	0.0	0.0	1.4
6	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.3
7	1.2	0.9	0.0	0.0	0.0	0.0	0.0	2.1
8	0.6	0.3	0.0	0.0	0.0	0.0	0.0	1.0
9	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.3
10	0.1	0.3	0.3	0.0	0.0	0.0	0.0	0.6
11	0.3	2.4	2.7	0.0	0.0	0.0	0.0	5.4
12	1.5	7.1	8.4	0.7	0.0	0.0	0.0	17.6
13	1.9	9.6	11.1	1.7	0.0	0.0	0.0	24.4
14	2.3	7.3	8.8	2.0	0.4	0.0	0.0	20.7
15	1.1	3.7	4.3	1.3	0.2	0.0	0.0	10.7
16	4.5	1.6	1.9	0.7	0.1	0.0	0.0	8.8
17	0.3	0.4	0.6	0.2	0.0	0.0	0.0	1.6
18	2.6	1.6	0.3	0.1	0.0	0.0	0.0	4.7
19	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.3
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	18.3	35.5	38.4	6.8	0.8	0.1	0.2	100.0

Table 1.4

Algae water 10 mg/L

C number	z number							
	0	2	4	6	8	10	12	% carbon no
5	3.5	0.0	0.0	0.0	0.0	0.0	0.0	3.5
6	1.4	0.0	0.0	0.0	0.0	0.0	0.0	1.4
7	3.2	2.5	0.0	0.0	0.0	0.0	0.0	5.7
8	2.1	1.1	0.0	0.0	0.0	0.0	0.0	3.2
9	0.1	1.3	0.0	0.0	0.0	0.0	0.0	1.4
10	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.5
11	0.2	1.9	2.2	0.0	0.0	0.0	0.0	4.4
12	1.6	6.2	7.6	0.6	0.0	0.0	0.0	16.1
13	1.8	8.9	10.5	1.7	0.0	0.0	0.0	22.9
14	2.3	6.8	8.5	1.9	0.4	0.0	0.0	20.0
15	0.9	3.3	4.1	1.3	0.2	0.0	0.0	9.8
16	3.3	1.2	1.7	0.6	0.1	0.0	0.0	6.9
17	0.1	0.2	0.5	0.2	0.0	0.0	0.0	1.0
18	1.8	0.6	0.1	0.1	0.0	0.0	0.0	2.8
19	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.2
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	22.6	34.4	35.4	6.5	0.8	0.1	0.1	100.0

Table 1.5

Algae water 20 mg/L

C number	z number							% carbon no
	0	2	4	6	8	10	12	
5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	1.5
6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.4
7	1.1	1.0	0.0	0.0	0.0	0.0	0.0	2.1
8	0.8	0.5	0.0	0.0	0.0	0.0	0.0	1.3
9	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.7
10	0.3	1.5	1.0	0.0	0.0	0.0	0.0	2.8
11	0.8	5.7	4.8	0.0	0.0	0.0	0.0	11.2
12	1.9	9.4	10.2	0.9	0.0	0.0	0.0	22.4
13	2.1	9.4	10.9	1.8	0.0	0.0	0.0	24.2
14	1.9	6.1	7.3	1.8	0.4	0.0	0.0	17.5
15	0.9	2.8	3.4	1.2	0.2	0.0	0.0	8.5
16	1.7	1.0	1.5	0.6	0.1	0.0	0.0	5.0
17	0.2	0.3	0.4	0.2	0.1	0.0	0.0	1.2
18	0.7	0.2	0.1	0.1	0.0	0.0	0.0	1.1
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	14.4	38.4	39.7	6.5	0.9	0.1	0.1	100.0

Table 1.6

Algae water 40 mg/L

C number	z number							% carbon no
	0	2	4	6	8	10	12	
5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	1.5
6	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5
7	1.1	0.9	0.0	0.0	0.0	0.0	0.0	2.1
8	0.7	0.5	0.0	0.0	0.0	0.0	0.0	1.3
9	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.8
10	0.4	1.9	1.3	0.0	0.0	0.0	0.0	3.5
11	1.0	7.1	5.9	0.0	0.0	0.0	0.0	14.1
12	2.1	11.3	11.7	1.0	0.0	0.0	0.0	26.1
13	1.9	9.6	11.2	1.8	0.0	0.0	0.0	24.5
14	1.4	4.8	6.2	1.6	0.5	0.0	0.0	14.4
15	0.5	1.7	2.3	0.9	0.3	0.0	0.0	5.8
16	1.4	0.6	0.9	0.4	0.1	0.1	0.0	3.5
17	0.1	0.2	0.3	0.1	0.1	0.0	0.0	0.8
18	0.6	0.2	0.1	0.0	0.0	0.0	0.0	1.1
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	13.6	39.4	39.9	5.9	1.0	0.2	0.1	100.0

Table 1.7

Algae water 80 mg/L

C number	z number							% carbon no
	0	2	4	6	8	10	12	
5	1.4	0.0	0.0	0.0	0.0	0.0	0.0	1.4
6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.4
7	0.9	0.8	0.0	0.0	0.0	0.0	0.0	1.7
8	0.6	0.5	0.0	0.0	0.0	0.0	0.0	1.1
9	0.2	0.7	0.0	0.0	0.0	0.0	0.0	0.9
10	0.5	3.1	1.8	0.0	0.0	0.0	0.0	5.4
11	1.4	10.4	8.4	0.0	0.0	0.0	0.0	20.2
12	2.1	13.1	14.6	1.2	0.0	0.0	0.0	31.0
13	1.4	8.0	11.0	1.9	0.0	0.0	0.0	22.3
14	0.8	2.9	4.4	1.3	0.5	0.0	0.0	9.8
15	0.3	0.9	1.3	0.5	0.2	0.0	0.0	3.2
16	0.7	0.3	0.4	0.2	0.1	0.1	0.0	1.7
17	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.3
18	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.5
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% by z No	11.0	40.7	42.1	5.2	0.8	0.1	0.0	100.0

The distributions of ions in selected samples were used to prepare a three-dimensional plots of the ion abundances for each n and Z value. The three-dimensional plot for the sample that contained 80 mg WAF/L Algae water is given in Figure 1.1 and the plot for the sample that contained 0 mg WAF/L (control Algae waters) is given in Figure 1.2.

Using the statistical method of Clemente et al. (2003), the data from Figure 1.1 compared to data from the analyses of samples of neat naphthenic acids previously supplied by API. There were no statistical differences between the naphthenic acids in these two samples.

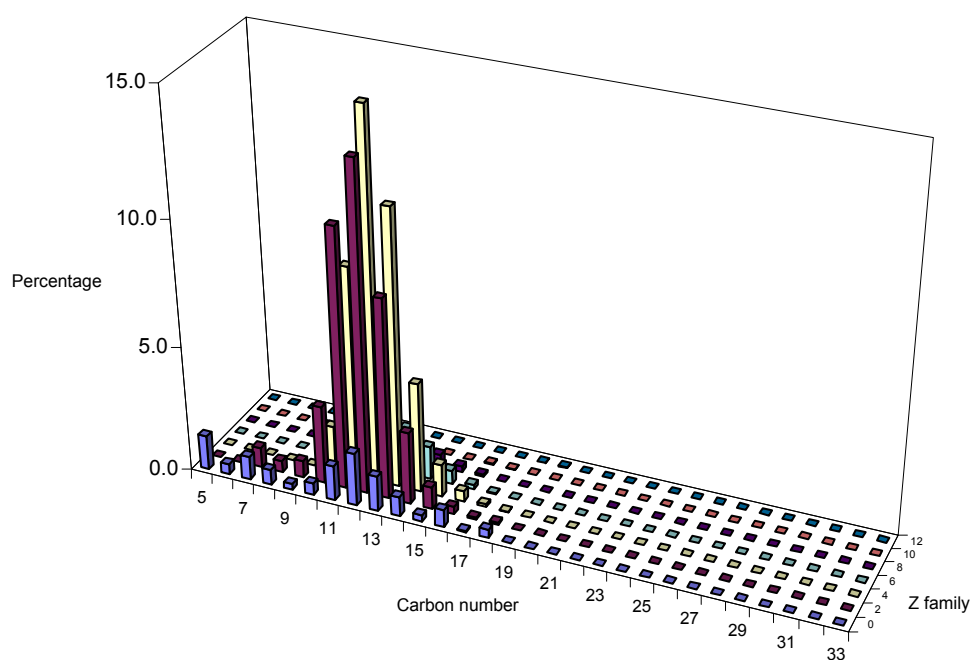


Figure. 1.1 . Three-dimensional plot of naphthenic acids in the Algae water that contained 80 mg WAF/L. The sum of all bars equals 100%.

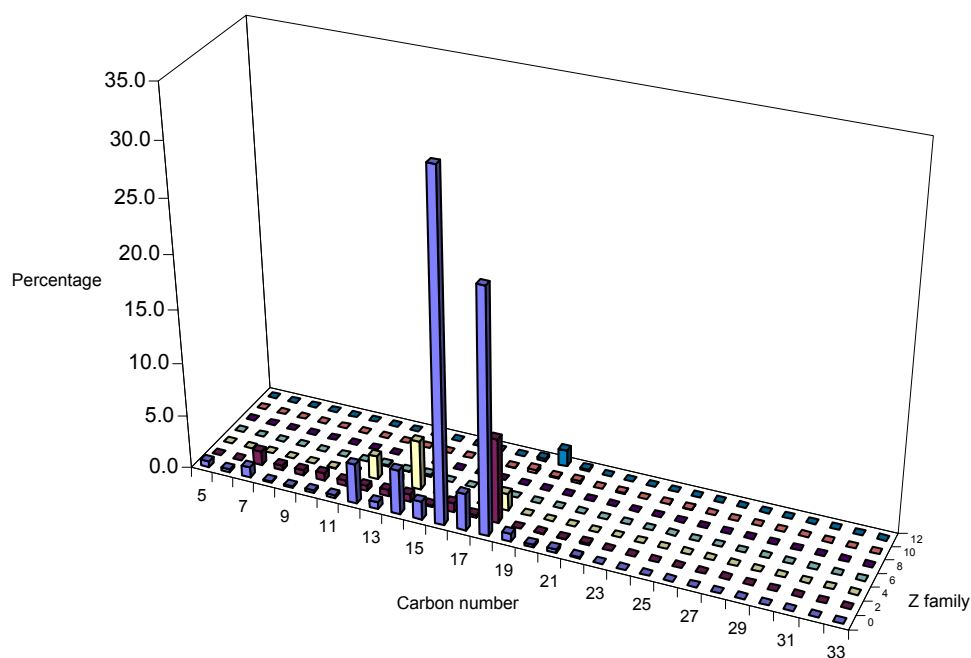


Figure. 1.2 . Three-dimensional plot of naphthenic acids in the Algae water that contained 0 mg WAF/L (control). The sum of all bars equals 100%.

The three-dimensional plot for the sample that contained 0 mg WAF/L (control) is given in Figure 1.2. This plot is very different from the plot of the naphthenic acids-containing sample (Figure 1.1). The most abundant ions in Figure 1.2 are those corresponding to $n = 16$, $Z = 0$ and $n = 18$, $Z = 0$. The most common fatty acids found in the phospholipids and glycolipids in cell membranes are C-16 (palmitic) and C-18 (stearic) acids (Stryer 1981). These fatty acids are also commonly found in the membranes of microorganisms (Lechevalier and Lechevalier 1988; O'Leary and Wilkinson 1988). Palmitic and stearic acids were observed by in bacterial cultures in which naphthenic acids had been removed by biodegradation (Clemente et al. 2004; Biryukova et al. 2007). In addition, these acids have also been found in river water samples (Fatoki and Vernon 1989; Scott et al. 2008). Thus, the appearance and predominance of palmitic and stearic acids in the 0 mg WAF/L (control) samples (Figure 1.2) is not unexpected.

The statistical method of Clemente et al. (2003), was also used to determine if there were any differences between the various samples of Algae water. This method allows two samples to be compared to one another. The 80 mg WAF/L samples were arbitrarily chosen for each set of comparisons. Thus, each Algae water sample was compared to the 80 mg WAF/L Algae water sample. The results of these comparisons are summarized in Table 1.8.

Table 1.8. Statistical comparisons of distributions of naphthenic acids in various Algae waters to the distributions of naphthenic acids in the Algae water that contained 80 mg WAF/L. "S" indicates a significant difference ($P < 0.05$); "NS" indicates no significant difference ($P > 0.05$).

Sample concentration (mg/L)	Group 1 (C ₅ to C ₁₃)	Group 2 (C ₁₄ to C ₂₁)	Group 3 (C ₂₂ to C ₃₃)
0 S		NS	S
2.5 NS		NS	NS
5 NS		S	S
10 NS		NS	NS
20 NS		NS	NS
40 NS		NS	NS

In this statistical method, the ion distributions from the GC-MS analysis are divided into three groups based on carbon numbers. These are: Group 1 (C₅ to C₁₃), Group 2 (C₁₄ to C₂₁), and Group 3 (C₂₂ to C₃₃), as shown in Table 1.8. The mean of the relative abundance of the ions in each group from one naphthenic acids sample is compared to the sum of the relative abundance of the ions in each group from another naphthenic acids sample by a two-sided t-test. For example, the comparisons in Table 1.8 are based on the relative abundance of ions in one group of an individual sample being compared to the relative abundance of the ions in the corresponding group in the 80 mg WAF/L Algae water sample.

Figures 1.1 and 1.2 show a noticeable difference between the distribution of ions in the 80 mg WAF/L Algae water sample and the 0 mg WAF/L Algae water sample. The statistical comparison of these two samples (Table 1.8) shows that the Group 1 naphthenic acids are statistically different and that the Group 3 naphthenic acids are statistically different. Surprisingly, no statistical difference was observed between the Group 2 naphthenic acids in the 80 mg WAF/L and 0 mg WAF/L samples. This result is similar to the observation with the fish water and the *Daphnia* water.

Another statistical difference observed (Table 1.8) indicated that the Group 2 naphthenic acids in the 5 mg WAF/L Algae water was different from the Group 2 naphthenic acids in the 80 mg WAF/L Algae water (Table 1.8). The reason for this is unclear. However, when the ion distribution from the 5 mg WAF/L Algae water sample was compared to the ion distributions from the other concentrations of WAF in the Algae waters (i.e. 2.5, 10, 20, and 40 mg/L), no statistically significant differences were observed.

The only other statistical difference shown in Tables 1.8 is Group 3 (C₂₂ to C₃₃) naphthenic acids of the 5 mg WAF/L Algae water sample. However, commercial naphthenic acids preparations typically lack Group 3 acids (Clemente et al. 2003). For example, only 0.002% of the ions detected in the 80 mg/L WAF/L Algae water sample extract fell into the Group 3 acids. In contrast, 0.000% of the ions detected in the 5 mg/L WAF/L Algae water sample extract fell into the Group 3 acids. Although this small difference was enough to be considered statistically different by the method of Clemente et al. (2003), the magnitude of the difference is not important.

Using the same statistical test, the distributions of naphthenic acids in the 80 mg WAF/L *Daphnia* water (Figure 1.1) were compared to the naphthenic acids in the 80 mg WAF/L Algae water (Figure 1.1). There were no significant differences between the relative abundances of the acids in Groups 1 or 2 in these two samples.

2.0 References

- Biryukova, O.V., P.M. Fedorak & S.A. Quideau. (2007) Biodegradation of naphthenic acids by rhizosphere microorganisms. *Chemosphere* 67, 2058-2064.
- Clemente, J.S, N.G.N. Prasad, M.D. MacKinnon & P.M. Fedorak. (2003) A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. *Chemosphere* 50, 1265-1274.
- Clemente, J.S., M.D. MacKinnon & P.M. Fedorak. (2004) Aerobic biodegradation of two commercial naphthenic acids preparations. *Environ. Sci. Technol.* 38, 1009-1016.
- Fatoki, O.S. & F. Vernon. (1989) Determination of free fatty acid content of polluted and unpolluted waters. *Water Res.* 23, 123–125.
- Holowenko, F.M., M.D. MacKinnon & P.M. Fedorak. (2002) Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* 36, 2843-2855.
- Lechevalier, H., & M.P. Lechevalier. (1988) Chemotaxonomic use of lipids – an overview. In: Ratledge, C., Wilkinson, S.G. (Eds.). In: *Microbial Lipids*, vol. 1. Academic Press, New York, NY, USA, pp. 869–902.
- Merlin, M., S.E. Guigard & P.M. Fedorak. (2007) Detection of naphthenic acids in water using gas chromatography-mass spectrometry. *J. Chromatogr. A.* 1140, 225-229.
- O'Leary, W.M. & S.G. Wilkinson. (1988) Gram positive bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.), In: *Microbial Lipids*, vol. 1. Academic Press, New York, NY, USA, pp. 117–201.
- Scott, A.C., R.F. Young & P.M. Fedorak. (2008) Comparison of GC-MS and FTIR methods for quantifying naphthenic acids in water samples. *Chemosphere* 73, 1258-1264.

Stryer, L. (1981) Biochemistry 2nd Edition. W,H. Freeman and Company, New York. p. 207.

Young, R.F., W.V. Wismer, & P.M. Fedorak. (2008) Estimating naphthenic acids in laboratory-exposed fish and in fish from the wild. *Chemosphere* 73, 498-505.

APPENDIX I – STATISTICS

1

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	3.1
24	CONTROL	B	3.3
24	CONTROL	C	3.0
24	CONTROL	D	3.2
24	CONTROL	E	3.3
24	CONTROL	F	3.6
24	LEVEL 1	A	3.2
24	LEVEL 1	B	3.2
24	LEVEL 1	C	3.6
24	LEVEL 2	A	2.7
24	LEVEL 2	B	3.6
24	LEVEL 2	C	3.6
24	LEVEL 3	A	3.9
24	LEVEL 3	B	2.8
24	LEVEL 3	C	2.7
24	LEVEL 4	A	2.9
24	LEVEL 4	B	2.6
24	LEVEL 4	C	3.1
24	LEVEL 5	A	1.7
24	LEVEL 5	B	1.4
24	LEVEL 5	C	1.6
24	LEVEL 6	A	1.1
24	LEVEL 6	B	1.1
24	LEVEL 6	C	1.3
48	CONTROL	A	12.0
48	CONTROL	B	13.0
48	CONTROL	C	13.0
48	CONTROL	D	14.0
48	CONTROL	E	13.0
48	CONTROL	F	14.0
48	LEVEL 1	A	13.0
48	LEVEL 1	B	12.0
48	LEVEL 1	C	12.0
48	LEVEL 2	A	12.0
48	LEVEL 2	B	15.0
48	LEVEL 2	C	12.0
48	LEVEL 3	A	12.0
48	LEVEL 3	B	10.0

2

ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
48	LEVEL 3	C	13.00
48	LEVEL 4	A	6.40
48	LEVEL 4	B	7.60
48	LEVEL 4	C	8.40
48	LEVEL 5	A	3.30
48	LEVEL 5	B	3.60
48	LEVEL 5	C	3.30
48	LEVEL 6	A	1.60
48	LEVEL 6	B	1.40
48	LEVEL 6	C	1.30
72	CONTROL	A	39.00
72	CONTROL	B	38.00
72	CONTROL	C	52.00
72	CONTROL	D	55.00
72	CONTROL	E	46.00
72	CONTROL	F	53.00
72	LEVEL 1	A	45.00
72	LEVEL 1	B	44.00
72	LEVEL 1	C	51.00
72	LEVEL 2	A	40.00
72	LEVEL 2	B	45.00
72	LEVEL 2	C	50.00
72	LEVEL 3	A	41.00
72	LEVEL 3	B	32.00
72	LEVEL 3	C	47.00
72	LEVEL 4	A	32.00
72	LEVEL 4	B	32.00
72	LEVEL 4	C	27.00
72	LEVEL 5	A	7.70
72	LEVEL 5	B	9.70
72	LEVEL 5	C	8.80
72	LEVEL 6	A	1.00
72	LEVEL 6	B	0.89
72	LEVEL 6	C	1.20
96	CONTROL	A	139.00
96	CONTROL	B	138.00
96	CONTROL	C	175.00
96	CONTROL	D	157.00
96	CONTROL	E	155.00
96	CONTROL	F	162.00
96	LEVEL 1	A	139.00
96	LEVEL 1	B	146.00
96	LEVEL 1	C	155.00
96	LEVEL 2	A	141.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOURL	TREATMNT	REP	CELL_104
96	LEVEL 2	B	158.0
96	LEVEL 2	C	153.0
96	LEVEL 3	A	165.0
96	LEVEL 3	B	133.0
96	LEVEL 3	C	150.0
96	LEVEL 4	A	125.0
96	LEVEL 4	B	104.0
96	LEVEL 4	C	94.0
96	LEVEL 5	A	20.0
96	LEVEL 5	B	20.0
96	LEVEL 5	C	25.0
96	LEVEL 6	A	1.0
96	LEVEL 6	B	1.0
96	LEVEL 6	C	1.6

N = 102

ABC LABORATORIES, INC. 4
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	24	CONTROL	A	0.047142
0	24	CONTROL	B	0.049747
0	24	CONTROL	C	0.045776
0	24	CONTROL	D	0.048465
0	24	CONTROL	E	0.049747
0	24	CONTROL	F	0.053372
0	24	LEVEL 1	A	0.048465
0	24	LEVEL 1	B	0.048465
0	24	LEVEL 1	C	0.053372
0	24	LEVEL 2	A	0.041385
0	24	LEVEL 2	B	0.053372
0	24	LEVEL 2	C	0.053372
0	24	LEVEL 3	A	0.056707
0	24	LEVEL 3	B	0.042901
0	24	LEVEL 3	C	0.041385
0	24	LEVEL 4	A	0.044363
0	24	LEVEL 4	B	0.039813
0	24	LEVEL 4	C	0.047142
0	24	LEVEL 5	A	0.022110
0	24	LEVEL 5	B	0.014020
0	24	LEVEL 5	C	0.019583
0	24	LEVEL 6	A	0.003971
0	24	LEVEL 6	B	0.003971
0	24	LEVEL 6	C	0.010932
0	48	CONTROL	A	0.051769
0	48	CONTROL	B	0.053436
0	48	CONTROL	C	0.053436
0	48	CONTROL	D	0.054980
0	48	CONTROL	E	0.053436
0	48	CONTROL	F	0.054980
0	48	LEVEL 1	A	0.053436
0	48	LEVEL 1	B	0.051769
0	48	LEVEL 1	C	0.051769
0	48	LEVEL 2	A	0.051769
0	48	LEVEL 2	B	0.056418
0	48	LEVEL 2	C	0.051769
0	48	LEVEL 3	A	0.051769
0	48	LEVEL 3	B	0.047971
0	48	LEVEL 3	C	0.053436
0	48	LEVEL 4	A	0.038673
0	48	LEVEL 4	B	0.042253
0	48	LEVEL 4	C	0.044338
0	48	LEVEL 5	A	0.024873
0	48	LEVEL 5	B	0.026686

5

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	48	LEVEL 5	C	0.024873
0	48	LEVEL 6	A	0.009792
0	48	LEVEL 6	B	0.007010
0	48	LEVEL 6	C	0.005466
0	72	CONTROL	A	0.050883
0	72	CONTROL	B	0.050522
0	72	CONTROL	C	0.054878
0	72	CONTROL	D	0.055657
0	72	CONTROL	E	0.053176
0	72	CONTROL	F	0.055143
0	72	LEVEL 1	A	0.052870
0	72	LEVEL 1	B	0.052558
0	72	LEVEL 1	C	0.054609
0	72	LEVEL 2	A	0.051234
0	72	LEVEL 2	B	0.052870
0	72	LEVEL 2	C	0.054334
0	72	LEVEL 3	A	0.051577
0	72	LEVEL 3	B	0.048135
0	72	LEVEL 3	C	0.053474
0	72	LEVEL 4	A	0.048135
0	72	LEVEL 4	B	0.048135
0	72	LEVEL 4	C	0.045776
0	72	LEVEL 5	A	0.028350
0	72	LEVEL 5	B	0.031557
0	72	LEVEL 5	C	0.030205
0	72	LEVEL 6	A	0.000000
0	72	LEVEL 6	B	-0.001619
0	72	LEVEL 6	C	0.002532
0	96	CONTROL	A	0.051401
0	96	CONTROL	B	0.051326
0	96	CONTROL	C	0.053800
0	96	CONTROL	D	0.052669
0	96	CONTROL	E	0.052536
0	96	CONTROL	F	0.052996
0	96	LEVEL 1	A	0.051401
0	96	LEVEL 1	B	0.051913
0	96	LEVEL 1	C	0.052536
0	96	LEVEL 2	A	0.051550
0	96	LEVEL 2	B	0.052735
0	96	LEVEL 2	C	0.052400
0	96	LEVEL 3	A	0.053187
0	96	LEVEL 3	B	0.050941
0	96	LEVEL 3	C	0.052194
0	96	LEVEL 4	A	0.050295

ABC LABORATORIES, INC. 6
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	96	LEVEL 4	B	0.048379
0	96	LEVEL 4	C	0.047326
0	96	LEVEL 5	A	0.031206
0	96	LEVEL 5	B	0.031206
0	96	LEVEL 5	C	0.033530
0	96	LEVEL 6	A	0.000000
0	96	LEVEL 6	B	0.000000
0	96	LEVEL 6	C	0.004896

N = 96

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.045776	0.053372	0.049041	.002621399
LEVEL 1	3	0.048465	0.053372	0.050100	.002833419
LEVEL 2	3	0.041385	0.053372	0.049377	.006920555
LEVEL 3	3	0.041385	0.056707	0.046998	.008442714
LEVEL 4	3	0.039813	0.047142	0.043773	.003699887
LEVEL 5	3	0.014020	0.022110	0.018571	.004138884
LEVEL 6	3	0.003971	0.010932	0.006291	.004018697

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	5.3453	0.046290	0.051792
LEVEL 1	5.6555	0.043062	0.057139
LEVEL 2	14.0158	0.032185	0.066568
LEVEL 3	17.9640	0.026025	0.067971
LEVEL 4	8.4525	0.034582	0.052964
LEVEL 5	22.2869	0.008289	0.028852
LEVEL 6	63.8755	-0.003692	0.016274

----- T1=0 TN=48 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.051769	0.054980	0.053673	.001200991
LEVEL 1	3	0.051769	0.053436	0.052325	.000962764
LEVEL 2	3	0.051769	0.056418	0.053318	.002684000
LEVEL 3	3	0.047971	0.053436	0.051059	.002801328
LEVEL 4	3	0.038673	0.044338	0.041755	.002865336
LEVEL 5	3	0.024873	0.026686	0.025478	.001046584

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	2.23760	0.052413	0.054934
LEVEL 1	1.83998	0.049933	0.054716
LEVEL 2	5.03390	0.046651	0.059986
LEVEL 3	5.48649	0.044100	0.058018
LEVEL 4	6.86231	0.034637	0.048873
LEVEL 5	4.10786	0.022878	0.028077

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=48 -----
 (continued)

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN
LEVEL 6	3	.005465922	.009791742	.007422501

TREATMNT	STD_DEV	CV	LOWER_CI	UPPER_CI
LEVEL 6	.002192236	29.5350	.001976685	0.012868

----- T1=0 TN=72 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.050522	0.055657	0.053377	.002235416
LEVEL 1	3	0.052558	0.054609	0.053346	.001104832
LEVEL 2	3	0.051234	0.054334	0.052813	.001550408
LEVEL 3	3	0.048135	0.053474	0.051062	.002706540
LEVEL 4	3	0.045776	0.048135	0.047349	.001362379
LEVEL 5	3	0.028350	0.031557	0.030037	.001610051
LEVEL 6	3	-0.001619	0.002532	0.000305	.002092079

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	4.188	0.051031	0.055722
LEVEL 1	2.071	0.050601	0.056090
LEVEL 2	2.936	0.048961	0.056664
LEVEL 3	5.300	0.044339	0.057786
LEVEL 4	2.877	0.043964	0.050733
LEVEL 5	5.360	0.026038	0.034037
LEVEL 6	686.889	-0.004892	0.005502

ABC LABORATORIES, INC. 9
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=96 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.051326	0.053800	0.052454	.000952946
LEVEL 1	3	0.051401	0.052536	0.051950	.000568363
LEVEL 2	3	0.051550	0.052735	0.052228	.000611306
LEVEL 3	3	0.050941	0.053187	0.052107	.001125406
LEVEL 4	3	0.047326	0.050295	0.048667	.001505222
LEVEL 5	3	0.031206	0.033530	0.031980	.001342000
LEVEL 6	3	0.000000	0.004896	0.001632	.002826633

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	1.817	0.051454	0.053455
LEVEL 1	1.094	0.050538	0.053362
LEVEL 2	1.170	0.050710	0.053747
LEVEL 3	2.160	0.049312	0.054903
LEVEL 4	3.093	0.044927	0.052406
LEVEL 5	4.196	0.028647	0.035314
LEVEL 6	173.205	-0.005390	0.008654

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	2.89	0.0398
TRANSFORMED	6	17	9.82	0.0001

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 11
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6
		Number of Observations Read 24
		Number of Observations Used 24

ABC LABORATORIES, INC. 12
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	774.666667	129.111111	5.99
Error	17	366.333333	21.549020	
Corrected Total	23	1141.000000		

Source	Pr > F
Model	0.0016
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.678937	37.13674	4.642092	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	774.666667	129.111111	5.99

Source	Pr > F
TREATMNT	0.0016

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Dunnnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	21.54902
Critical Value of Dunnnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	1.167	-8.365	10.698	
LEVEL 2 - CONTROL	0.500	-9.031	10.031	
LEVEL 3 - CONTROL	-2.500	-12.031	7.031	
LEVEL 4 - CONTROL	-6.167	-15.698	3.365	
LEVEL 5 - CONTROL	-11.667	-21.198	-2.135	***
LEVEL 6 - CONTROL	-14.667	-24.198	-5.135	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.049	0.003		
LEVEL 1	3	0.050	0.003	0.9990	
LEVEL 2	3	0.049	0.007	1.0000	
LEVEL 3	3	0.047	0.008	0.9527	
LEVEL 4	3	0.044	0.004	0.3222	
LEVEL 5	3	0.019	0.004	0.0130	*
LEVEL 6	3	0.006	0.004	0.0019	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 4.6421.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 48

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.68	0.1866
TRANSFORMED	6	17	4.57	0.0062

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 16
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=48 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 17
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=48 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	926.791667	154.465278	13.49
Error	17	194.708333	11.453431	
Corrected Total	23	1121.500000		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.826386	27.07433	3.384292	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	926.7916667	154.4652778	13.49

Source	Pr > F
TREATMNT	<.0001

ABC LABORATORIES, INC. 18
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=48 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	11.45343
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-2.250	-9.199	4.699	
LEVEL 1 - CONTROL	-3.917	-10.865	3.032	
LEVEL 3 - CONTROL	-5.083	-12.032	1.865	
LEVEL 4 - CONTROL	-11.250	-18.199	-4.301	***
LEVEL 5 - CONTROL	-14.250	-21.199	-7.301	***
LEVEL 6 - CONTROL	-17.250	-24.199	-10.301	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 48

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.054	0.001		
LEVEL 1	3	0.052	0.001	0.4573	
LEVEL 2	3	0.053	0.003	0.8879	
LEVEL 3	3	0.051	0.003	0.2162	
LEVEL 4	3	0.042	0.003	0.0012	*
LEVEL 5	3	0.025	0.001	0.0001	*
LEVEL 6	3	0.007	0.002	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.3843.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

ABC LABORATORIES, INC. 20
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 72

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	<0.01	<0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	0.85	0.5475
TRANSFORMED	6	17	0.84	0.5585

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 21
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=72 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 22
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=72 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	885.500000	147.583333	9.58
Error	17	262.000000	15.411765	
Corrected Total	23	1147.500000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.771678	31.40626	3.925782	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	885.5000000	147.5833333	9.58

Source	Pr > F
TREATMNT	0.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=72 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.41176
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	-0.833	-8.894	7.227	
LEVEL 2 - CONTROL	-1.833	-9.894	6.227	
LEVEL 3 - CONTROL	-4.333	-12.394	3.727	
LEVEL 4 - CONTROL	-10.000	-18.061	-1.939	***
LEVEL 5 - CONTROL	-13.333	-21.394	-5.273	***
LEVEL 6 - CONTROL	-16.333	-24.394	-8.273	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 72

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.053	0.002		
LEVEL 1	3	0.053	0.001	0.9996	
LEVEL 2	3	0.053	0.002	0.9753	
LEVEL 3	3	0.051	0.003	0.5048	
LEVEL 4	3	0.047	0.001	0.0118	*
LEVEL 5	3	0.030	0.002	0.0009	*
LEVEL 6	3	0.000	0.002	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9258.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

ABC LABORATORIES, INC. 25
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.37	0.0225
TRANSFORMED	6	17	3.67	0.0159

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 26
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 27
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	880.000000	146.666667	9.30
Error	17	268.000000	15.764706	
Corrected Total	23	1148.000000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.766551	31.76383	3.970479	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	880.000000	146.666667	9.30

Source	Pr > F
TREATMNT	0.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.76471
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-0.667	-8.819	7.486	
LEVEL 3 - CONTROL	-1.667	-9.819	6.486	
LEVEL 1 - CONTROL	-2.667	-10.819	5.486	
LEVEL 4 - CONTROL	-10.000	-18.152	-1.848	***
LEVEL 5 - CONTROL	-13.000	-21.152	-4.848	***
LEVEL 6 - CONTROL	-16.000	-24.152	-7.848	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.052	0.001		
LEVEL 1	3	0.052	0.001	0.8835	
LEVEL 2	3	0.052	0.001	0.9999	
LEVEL 3	3	0.052	0.001	0.9852	
LEVEL 4	3	0.049	0.002	0.0128	*
LEVEL 5	3	0.032	0.001	0.0013	*
LEVEL 6	3	0.002	0.003	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9705.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

ABC LABORATORIES, INC. 30
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=0 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0	A	1
CONTROL	0	B	1
CONTROL	0	C	1
CONTROL	0	D	1
CONTROL	0	E	1
CONTROL	0	F	1

N = 6

----- HOUR=24 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.0	A	3.1
CONTROL	0.0	B	3.3
CONTROL	0.0	C	3.0
CONTROL	0.0	D	3.2
CONTROL	0.0	E	3.3
CONTROL	0.0	F	3.6
LEVEL 1	2.5	A	3.2
LEVEL 1	2.5	B	3.2
LEVEL 1	2.5	C	3.6
LEVEL 2	5.0	A	2.7
LEVEL 2	5.0	B	3.6
LEVEL 2	5.0	C	3.6
LEVEL 3	10.0	A	3.9
LEVEL 3	10.0	B	2.8
LEVEL 3	10.0	C	2.7
LEVEL 4	20.0	A	2.9
LEVEL 4	20.0	B	2.6
LEVEL 4	20.0	C	3.1
LEVEL 5	40.0	A	1.7
LEVEL 5	40.0	B	1.4
LEVEL 5	40.0	C	1.6
LEVEL 6	80.0	A	1.1
LEVEL 6	80.0	B	1.1
LEVEL 6	80.0	C	1.3

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=48 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.0	A	12.0
CONTROL	0.0	B	13.0
CONTROL	0.0	C	13.0
CONTROL	0.0	D	14.0
CONTROL	0.0	E	13.0
CONTROL	0.0	F	14.0
LEVEL 1	2.5	A	13.0
LEVEL 1	2.5	B	12.0
LEVEL 1	2.5	C	12.0
LEVEL 2	5.0	A	12.0
LEVEL 2	5.0	B	15.0
LEVEL 2	5.0	C	12.0
LEVEL 3	10.0	A	12.0
LEVEL 3	10.0	B	10.0
LEVEL 3	10.0	C	13.0
LEVEL 4	20.0	A	6.4
LEVEL 4	20.0	B	7.6
LEVEL 4	20.0	C	8.4
LEVEL 5	40.0	A	3.3
LEVEL 5	40.0	B	3.6
LEVEL 5	40.0	C	3.3
LEVEL 6	80.0	A	1.6
LEVEL 6	80.0	B	1.4
LEVEL 6	80.0	C	1.3

N = 24

----- HOUR=72 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0	A	39
CONTROL	0	B	38
CONTROL	0	C	52
CONTROL	0	D	55
CONTROL	0	E	46
CONTROL	0	F	53

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=72 -----
 (continued)

TREATMNT	CONC	REP	CELL_104
LEVEL 1	2.5	A	45.00
LEVEL 1	2.5	B	44.00
LEVEL 1	2.5	C	51.00
LEVEL 2	5.0	A	40.00
LEVEL 2	5.0	B	45.00
LEVEL 2	5.0	C	50.00
LEVEL 3	10.0	A	41.00
LEVEL 3	10.0	B	32.00
LEVEL 3	10.0	C	47.00
LEVEL 4	20.0	A	32.00
LEVEL 4	20.0	B	32.00
LEVEL 4	20.0	C	27.00
LEVEL 5	40.0	A	7.70
LEVEL 5	40.0	B	9.70
LEVEL 5	40.0	C	8.80
LEVEL 6	80.0	A	1.00
LEVEL 6	80.0	B	0.89
LEVEL 6	80.0	C	1.20

N = 24

----- HOUR=96 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.0	A	139
CONTROL	0.0	B	138
CONTROL	0.0	C	175
CONTROL	0.0	D	157
CONTROL	0.0	E	155
CONTROL	0.0	F	162
LEVEL 1	2.5	A	139
LEVEL 1	2.5	B	146
LEVEL 1	2.5	C	155
LEVEL 2	5.0	A	141
LEVEL 2	5.0	B	158

ABC LABORATORIES, INC. 33
SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=96 -----
(continued)

TREATMNT	CONC	REP	CELL_104
LEVEL 2	5	C	153.0
LEVEL 3	10	A	165.0
LEVEL 3	10	B	133.0
LEVEL 3	10	C	150.0
LEVEL 4	20	A	125.0
LEVEL 4	20	B	104.0
LEVEL 4	20	C	94.0
LEVEL 5	40	A	20.0
LEVEL 5	40	B	20.0
LEVEL 5	40	C	25.0
LEVEL 6	80	A	1.0
LEVEL 6	80	B	1.0
LEVEL 6	80	C	1.6

N = 24

ABC LABORATORIES, INC. 34
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=24 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.0	A	0.047142	0.049041	3.9
CONTROL	0.0	B	0.049747	0.049041	-1.3
CONTROL	0.0	C	0.045776	0.049041	6.7
CONTROL	0.0	D	0.048465	0.049041	1.2
CONTROL	0.0	E	0.049747	0.049041	-1.3
CONTROL	0.0	F	0.053372	0.049041	-8.7
LEVEL 1	2.5	A	0.048465	0.049041	1.2
LEVEL 1	2.5	B	0.048465	0.049041	1.2
LEVEL 1	2.5	C	0.053372	0.049041	-8.7
LEVEL 2	5.0	A	0.041385	0.049041	15.6
LEVEL 2	5.0	B	0.053372	0.049041	-8.7
LEVEL 2	5.0	C	0.053372	0.049041	-8.7
LEVEL 3	10.0	A	0.056707	0.049041	-15.5
LEVEL 3	10.0	B	0.042901	0.049041	12.5
LEVEL 3	10.0	C	0.041385	0.049041	15.6
LEVEL 4	20.0	A	0.044363	0.049041	9.5
LEVEL 4	20.0	B	0.039813	0.049041	18.8
LEVEL 4	20.0	C	0.047142	0.049041	3.9
LEVEL 5	40.0	A	0.022110	0.049041	54.9
LEVEL 5	40.0	B	0.014020	0.049041	71.4
LEVEL 5	40.0	C	0.019583	0.049041	60.1
LEVEL 6	80.0	A	0.003971	0.049041	91.9
LEVEL 6	80.0	B	0.003971	0.049041	91.9
LEVEL 6	80.0	C	0.010932	0.049041	77.7

N = 24

ABC LABORATORIES, INC. 35
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=48 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.0	A	0.051769	0.053673	3.5
CONTROL	0.0	B	0.053436	0.053673	0.4
CONTROL	0.0	C	0.053436	0.053673	0.4
CONTROL	0.0	D	0.054980	0.053673	-2.3
CONTROL	0.0	E	0.053436	0.053673	0.4
CONTROL	0.0	F	0.054980	0.053673	-2.3
LEVEL 1	2.5	A	0.053436	0.053673	0.4
LEVEL 1	2.5	B	0.051769	0.053673	3.5
LEVEL 1	2.5	C	0.051769	0.053673	3.5
LEVEL 2	5.0	A	0.051769	0.053673	3.5
LEVEL 2	5.0	B	0.056418	0.053673	-5.0
LEVEL 2	5.0	C	0.051769	0.053673	3.5
LEVEL 3	10.0	A	0.051769	0.053673	3.5
LEVEL 3	10.0	B	0.047971	0.053673	10.6
LEVEL 3	10.0	C	0.053436	0.053673	0.4
LEVEL 4	20.0	A	0.038673	0.053673	27.9
LEVEL 4	20.0	B	0.042253	0.053673	21.3
LEVEL 4	20.0	C	0.044338	0.053673	17.4
LEVEL 5	40.0	A	0.024873	0.053673	53.7
LEVEL 5	40.0	B	0.026686	0.053673	50.3
LEVEL 5	40.0	C	0.024873	0.053673	53.7
LEVEL 6	80.0	A	0.009792	0.053673	81.8
LEVEL 6	80.0	B	0.007010	0.053673	86.9
LEVEL 6	80.0	C	0.005466	0.053673	89.8

N = 24

ABC LABORATORIES, INC. 36
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=72 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.0	A	0.050883	0.053377	4.7
CONTROL	0.0	B	0.050522	0.053377	5.3
CONTROL	0.0	C	0.054878	0.053377	-2.7
CONTROL	0.0	D	0.055657	0.053377	-4.2
CONTROL	0.0	E	0.053176	0.053377	0.4
CONTROL	0.0	F	0.055143	0.053377	-3.2
LEVEL 1	2.5	A	0.052870	0.053377	0.9
LEVEL 1	2.5	B	0.052558	0.053377	1.5
LEVEL 1	2.5	C	0.054609	0.053377	-2.2
LEVEL 2	5.0	A	0.051234	0.053377	4.0
LEVEL 2	5.0	B	0.052870	0.053377	0.9
LEVEL 2	5.0	C	0.054334	0.053377	-1.7
LEVEL 3	10.0	A	0.051577	0.053377	3.4
LEVEL 3	10.0	B	0.048135	0.053377	9.8
LEVEL 3	10.0	C	0.053474	0.053377	-0.1
LEVEL 4	20.0	A	0.048135	0.053377	9.8
LEVEL 4	20.0	B	0.048135	0.053377	9.8
LEVEL 4	20.0	C	0.045776	0.053377	14.2
LEVEL 5	40.0	A	0.028350	0.053377	46.9
LEVEL 5	40.0	B	0.031557	0.053377	40.9
LEVEL 5	40.0	C	0.030205	0.053377	43.4
LEVEL 6	80.0	A	0.000000	0.053377	100.0
LEVEL 6	80.0	B	-0.001619	0.053377	103.0
LEVEL 6	80.0	C	0.002532	0.053377	95.3

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=96 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER__INH
CONTROL	0.0	A	0.051401	0.052454	2.0
CONTROL	0.0	B	0.051326	0.052454	2.2
CONTROL	0.0	C	0.053800	0.052454	-2.5
CONTROL	0.0	D	0.052669	0.052454	-0.3
CONTROL	0.0	E	0.052536	0.052454	-0.1
CONTROL	0.0	F	0.052996	0.052454	-0.9
LEVEL 1	2.5	A	0.051401	0.052454	2.0
LEVEL 1	2.5	B	0.051913	0.052454	1.0
LEVEL 1	2.5	C	0.052536	0.052454	-0.1
LEVEL 2	5.0	A	0.051550	0.052454	1.7
LEVEL 2	5.0	B	0.052735	0.052454	-0.4
LEVEL 2	5.0	C	0.052400	0.052454	0.1
LEVEL 3	10.0	A	0.053187	0.052454	-1.3
LEVEL 3	10.0	B	0.050941	0.052454	2.9
LEVEL 3	10.0	C	0.052194	0.052454	0.5
LEVEL 4	20.0	A	0.050295	0.052454	4.1
LEVEL 4	20.0	B	0.048379	0.052454	7.8
LEVEL 4	20.0	C	0.047326	0.052454	9.8
LEVEL 5	40.0	A	0.031206	0.052454	40.5
LEVEL 5	40.0	B	0.031206	0.052454	40.5
LEVEL 5	40.0	C	0.033530	0.052454	36.1
LEVEL 6	80.0	A	0.000000	0.052454	100.0
LEVEL 6	80.0	B	0.000000	0.052454	100.0
LEVEL 6	80.0	C	0.004896	0.052454	90.7

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-8.7	6.7	0.0	5.3	-5.5	5.6
LEVEL 1	2.5	3	-8.7	1.2	-2.1	5.8	-16.4	12.2
LEVEL 2	5.0	3	-8.7	15.6	-0.6	14.1	-35.6	34.4
LEVEL 3	10.0	3	-15.5	15.6	4.2	17.2	-38.5	46.9
LEVEL 4	20.0	3	3.9	18.8	10.7	7.5	-7.9	29.5
LEVEL 5	40.0	3	54.9	71.4	62.1	8.4	41.2	83.1
LEVEL 6	80.0	3	77.7	91.9	87.2	8.2	66.8	107.5

----- T1=0 TN=48 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-2.3	3.5	0.0	2.2	-2.2	2.3
LEVEL 1	2.5	3	0.4	3.5	2.5	1.8	-1.8	7.0
LEVEL 2	5.0	3	-5.0	3.5	0.7	5.0	-11.7	13.1
LEVEL 3	10.0	3	0.4	10.6	4.9	5.2	-8.0	17.8
LEVEL 4	20.0	3	17.4	27.9	22.2	5.3	8.9	35.5
LEVEL 5	40.0	3	50.3	53.7	52.5	1.9	47.7	57.4
LEVEL 6	80.0	3	81.8	89.8	86.2	4.1	76.0	96.3

----- T1=0 TN=72 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-4.2	5.3	0.0	4.2	-4.3	4.4
LEVEL 1	2.5	3	-2.2	1.5	0.1	2.1	-5.0	5.2
LEVEL 2	5.0	3	-1.7	4.0	1.1	2.9	-6.1	8.3
LEVEL 3	10.0	3	-0.1	9.8	4.3	5.1	-8.2	16.9
LEVEL 4	20.0	3	9.8	14.2	11.3	2.6	5.0	17.6
LEVEL 5	40.0	3	40.9	46.9	43.7	3.0	36.2	51.2
LEVEL 6	80.0	3	95.3	103.0	99.4	3.9	89.7	109.2

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=96 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-2.5	2.2	0.0	1.8	-1.8	1.9
LEVEL 1	2.5	3	-0.1	2.0	1.0	1.1	-1.6	3.7
LEVEL 2	5.0	3	-0.4	1.7	0.4	1.2	-2.4	3.3
LEVEL 3	10.0	3	-1.3	2.9	0.7	2.1	-4.6	6.0
LEVEL 4	20.0	3	4.1	9.8	7.2	2.9	0.1	14.3
LEVEL 5	40.0	3	36.1	40.5	39.0	2.6	32.7	45.4
LEVEL 6	80.0	3	90.7	100.0	96.9	5.4	83.5	110.3

ABC LABORATORIES, INC. 40
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

ESTIMATES OF COEFFICIENTS:
 SLOPE= 2.64 AND EC50= 34.87278758

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 200.71 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 10.35
 R-SQUARED= 92.60

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 11.432213392 95% LOWER LIMIT= 7.155287415 95% UPPER LIMIT= 15.709139368
PERCENT= 10	EC= 15.172114422 95% LOWER LIMIT= 10.641311279 95% UPPER LIMIT= 19.702917565
PERCENT= 20	EC= 20.627332255 95% LOWER LIMIT= 16.101052915 95% UPPER LIMIT= 25.153611595
PERCENT= 50	EC= 34.87278758 95% LOWER LIMIT= 31.058509645 95% UPPER LIMIT= 38.687065514
PERCENT= 90	EC= 80.15437267 , GREATER THAN HIGHEST CONC.= 80 95% LOWER LIMIT= 63.655162261 95% UPPER LIMIT= 96.653583078

ABC LABORATORIES, INC. 41
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 48

ESTIMATES OF COEFFICIENTS:
 SLOPE= 2.31 AND EC50= 37.371307448

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 1083.7 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 4.05
 R-SQUARED= 98.50

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 10.447382016 95% LOWER LIMIT= 8.6896175833 95% UPPER LIMIT= 12.205146448
PERCENT= 10	EC= 14.437026896 95% LOWER LIMIT= 12.504319758 95% UPPER LIMIT= 16.369734034
PERCENT= 20	EC= 20.508132555 95% LOWER LIMIT= 18.501044397 95% UPPER LIMIT= 22.515220714
PERCENT= 50	EC= 37.371307448 95% LOWER LIMIT= 35.55031302 95% UPPER LIMIT= 39.192301876
PERCENT= 90	EC= 96.73838183 , GREATER THAN HIGHEST CONC.= 80 95% LOWER LIMIT= 87.457057323 95% UPPER LIMIT= 106.01970634

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 72

ESTIMATES OF COEFFICIENTS:
 SLOPE= 7.75 AND EC50= 41.323320447

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 650.04 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 5.90
 R-SQUARED= 97.60

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 28.260149674 95% LOWER LIMIT= 22.238608158 95% UPPER LIMIT= 34.28169119
PERCENT= 10	EC= 31.120889768 95% LOWER LIMIT= 26.314158482 95% UPPER LIMIT= 35.927621054
PERCENT= 20	EC= 34.554172627 95% LOWER LIMIT= 31.404923691 95% UPPER LIMIT= 37.703421562
PERCENT= 50	EC= 41.323320447 95% LOWER LIMIT= 40.305244733 95% UPPER LIMIT= 42.341396161
PERCENT= 90	EC= 54.870436721 95% LOWER LIMIT= 44.285350731 95% UPPER LIMIT= 65.455522711

ABC LABORATORIES, INC. 43
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

ESTIMATES OF COEFFICIENTS:
 SLOPE= 5.59 AND EC50= 43.316910093

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 1686.2 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 3.63
 R-SQUARED= 99.10

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 25.571718356 95% LOWER LIMIT= 22.885609368 95% UPPER LIMIT= 28.257827344
PERCENT= 10	EC= 29.231262035 95% LOWER LIMIT= 26.950775707 95% UPPER LIMIT= 31.511748362
PERCENT= 20	EC= 33.797799037 95% LOWER LIMIT= 32.089588986 95% UPPER LIMIT= 35.506009087
PERCENT= 50	EC= 43.316910093 95% LOWER LIMIT= 41.984471462 95% UPPER LIMIT= 44.649348725
PERCENT= 90	EC= 64.189999659 95% LOWER LIMIT= 57.820234566 95% UPPER LIMIT= 70.559764752

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK ON: 13OCT09 *MR*
13 Oct 09

THE ANALYSIS WAS REVIEWED

BY: *WJG* ON: 22 Oct 09

ABC LABORATORIES, INC. 1
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	3.1
24	CONTROL	B	3.3
24	CONTROL	C	3.0
24	CONTROL	D	3.2
24	CONTROL	E	3.3
24	CONTROL	F	3.6
24	LEVEL 1	A	3.2
24	LEVEL 1	B	3.2
24	LEVEL 1	C	3.6
24	LEVEL 2	A	2.7
24	LEVEL 2	B	3.6
24	LEVEL 2	C	3.6
24	LEVEL 3	A	3.9
24	LEVEL 3	B	2.8
24	LEVEL 3	C	2.7
24	LEVEL 4	A	2.9
24	LEVEL 4	B	2.6
24	LEVEL 4	C	3.1
24	LEVEL 5	A	1.7
24	LEVEL 5	B	1.4
24	LEVEL 5	C	1.6
24	LEVEL 6	A	1.1
24	LEVEL 6	B	1.1
24	LEVEL 6	C	1.3
48	CONTROL	A	12.0
48	CONTROL	B	13.0
48	CONTROL	C	13.0
48	CONTROL	D	14.0
48	CONTROL	E	13.0
48	CONTROL	F	14.0
48	LEVEL 1	A	13.0
48	LEVEL 1	B	12.0
48	LEVEL 1	C	12.0
48	LEVEL 2	A	12.0
48	LEVEL 2	B	15.0
48	LEVEL 2	C	12.0
48	LEVEL 3	A	12.0
48	LEVEL 3	B	10.0

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOURL	TREATMNT	REP	CELL_104
48	LEVEL 3	C	13.00
48	LEVEL 4	A	6.40
48	LEVEL 4	B	7.60
48	LEVEL 4	C	8.40
48	LEVEL 5	A	3.30
48	LEVEL 5	B	3.60
48	LEVEL 5	C	3.30
48	LEVEL 6	A	1.60
48	LEVEL 6	B	1.40
48	LEVEL 6	C	1.30
72	CONTROL	A	39.00
72	CONTROL	B	38.00
72	CONTROL	C	52.00
72	CONTROL	D	55.00
72	CONTROL	E	46.00
72	CONTROL	F	53.00
72	LEVEL 1	A	45.00
72	LEVEL 1	B	44.00
72	LEVEL 1	C	51.00
72	LEVEL 2	A	40.00
72	LEVEL 2	B	45.00
72	LEVEL 2	C	50.00
72	LEVEL 3	A	41.00
72	LEVEL 3	B	32.00
72	LEVEL 3	C	47.00
72	LEVEL 4	A	32.00
72	LEVEL 4	B	32.00
72	LEVEL 4	C	27.00
72	LEVEL 5	A	7.70
72	LEVEL 5	B	9.70
72	LEVEL 5	C	8.80
72	LEVEL 6	A	1.00
72	LEVEL 6	B	0.89
72	LEVEL 6	C	1.20
96	CONTROL	A	139.00
96	CONTROL	B	138.00
96	CONTROL	C	175.00
96	CONTROL	D	157.00
96	CONTROL	E	155.00
96	CONTROL	F	162.00
96	LEVEL 1	A	139.00
96	LEVEL 1	B	146.00
96	LEVEL 1	C	155.00
96	LEVEL 2	A	141.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOURL	TREATMNT	REP	CELL_104
96	LEVEL 2	B	158.0
96	LEVEL 2	C	153.0
96	LEVEL 3	A	165.0
96	LEVEL 3	B	133.0
96	LEVEL 3	C	150.0
96	LEVEL 4	A	125.0
96	LEVEL 4	B	104.0
96	LEVEL 4	C	94.0
96	LEVEL 5	A	20.0
96	LEVEL 5	B	20.0
96	LEVEL 5	C	25.0
96	LEVEL 6	A	1.0
96	LEVEL 6	B	1.0
96	LEVEL 6	C	1.6

N = 102

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	24	CONTROL	A	0.047142
0	24	CONTROL	B	0.049747
0	24	CONTROL	C	0.045776
0	24	CONTROL	D	0.048465
0	24	CONTROL	E	0.049747
0	24	CONTROL	F	0.053372
0	24	LEVEL 1	A	0.048465
0	24	LEVEL 1	B	0.048465
0	24	LEVEL 1	C	0.053372
0	24	LEVEL 2	A	0.041385
0	24	LEVEL 2	B	0.053372
0	24	LEVEL 2	C	0.053372
0	24	LEVEL 3	A	0.056707
0	24	LEVEL 3	B	0.042901
0	24	LEVEL 3	C	0.041385
0	24	LEVEL 4	A	0.044363
0	24	LEVEL 4	B	0.039813
0	24	LEVEL 4	C	0.047142
0	24	LEVEL 5	A	0.022110
0	24	LEVEL 5	B	0.014020
0	24	LEVEL 5	C	0.019583
0	24	LEVEL 6	A	0.003971
0	24	LEVEL 6	B	0.003971
0	24	LEVEL 6	C	0.010932
0	48	CONTROL	A	0.051769
0	48	CONTROL	B	0.053436
0	48	CONTROL	C	0.053436
0	48	CONTROL	D	0.054980
0	48	CONTROL	E	0.053436
0	48	CONTROL	F	0.054980
0	48	LEVEL 1	A	0.053436
0	48	LEVEL 1	B	0.051769
0	48	LEVEL 1	C	0.051769
0	48	LEVEL 2	A	0.051769
0	48	LEVEL 2	B	0.056418
0	48	LEVEL 2	C	0.051769
0	48	LEVEL 3	A	0.051769
0	48	LEVEL 3	B	0.047971
0	48	LEVEL 3	C	0.053436
0	48	LEVEL 4	A	0.038673
0	48	LEVEL 4	B	0.042253
0	48	LEVEL 4	C	0.044338
0	48	LEVEL 5	A	0.024873
0	48	LEVEL 5	B	0.026686

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	48	LEVEL 5	C	0.024873
0	48	LEVEL 6	A	0.009792
0	48	LEVEL 6	B	0.007010
0	48	LEVEL 6	C	0.005466
0	72	CONTROL	A	0.050883
0	72	CONTROL	B	0.050522
0	72	CONTROL	C	0.054878
0	72	CONTROL	D	0.055657
0	72	CONTROL	E	0.053176
0	72	CONTROL	F	0.055143
0	72	LEVEL 1	A	0.052870
0	72	LEVEL 1	B	0.052558
0	72	LEVEL 1	C	0.054609
0	72	LEVEL 2	A	0.051234
0	72	LEVEL 2	B	0.052870
0	72	LEVEL 2	C	0.054334
0	72	LEVEL 3	A	0.051577
0	72	LEVEL 3	B	0.048135
0	72	LEVEL 3	C	0.053474
0	72	LEVEL 4	A	0.048135
0	72	LEVEL 4	B	0.048135
0	72	LEVEL 4	C	0.045776
0	72	LEVEL 5	A	0.028350
0	72	LEVEL 5	B	0.031557
0	72	LEVEL 5	C	0.030205
0	72	LEVEL 6	A	0.000000
0	72	LEVEL 6	B	-0.001619
0	72	LEVEL 6	C	0.002532
0	96	CONTROL	A	0.051401
0	96	CONTROL	B	0.051326
0	96	CONTROL	C	0.053800
0	96	CONTROL	D	0.052669
0	96	CONTROL	E	0.052536
0	96	CONTROL	F	0.052996
0	96	LEVEL 1	A	0.051401
0	96	LEVEL 1	B	0.051913
0	96	LEVEL 1	C	0.052536
0	96	LEVEL 2	A	0.051550
0	96	LEVEL 2	B	0.052735
0	96	LEVEL 2	C	0.052400
0	96	LEVEL 3	A	0.053187
0	96	LEVEL 3	B	0.050941
0	96	LEVEL 3	C	0.052194
0	96	LEVEL 4	A	0.050295

ABC LABORATORIES, INC. 6
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	96	LEVEL 4	B	0.048379
0	96	LEVEL 4	C	0.047326
0	96	LEVEL 5	A	0.031206
0	96	LEVEL 5	B	0.031206
0	96	LEVEL 5	C	0.033530
0	96	LEVEL 6	A	0.000000
0	96	LEVEL 6	B	0.000000
0	96	LEVEL 6	C	0.004896

N = 96

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.045776	0.053372	0.049041	.002621399
LEVEL 1	3	0.048465	0.053372	0.050100	.002833419
LEVEL 2	3	0.041385	0.053372	0.049377	.006920555
LEVEL 3	3	0.041385	0.056707	0.046998	.008442714
LEVEL 4	3	0.039813	0.047142	0.043773	.003699887
LEVEL 5	3	0.014020	0.022110	0.018571	.004138884
LEVEL 6	3	0.003971	0.010932	0.006291	.004018697

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	5.3453	0.046290	0.051792
LEVEL 1	5.6555	0.043062	0.057139
LEVEL 2	14.0158	0.032185	0.066568
LEVEL 3	17.9640	0.026025	0.067971
LEVEL 4	8.4525	0.034582	0.052964
LEVEL 5	22.2869	0.008289	0.028852
LEVEL 6	63.8755	-0.003692	0.016274

----- T1=0 TN=48 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.051769	0.054980	0.053673	.001200991
LEVEL 1	3	0.051769	0.053436	0.052325	.000962764
LEVEL 2	3	0.051769	0.056418	0.053318	.002684000
LEVEL 3	3	0.047971	0.053436	0.051059	.002801328
LEVEL 4	3	0.038673	0.044338	0.041755	.002865336
LEVEL 5	3	0.024873	0.026686	0.025478	.001046584

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	2.23760	0.052413	0.054934
LEVEL 1	1.83998	0.049933	0.054716
LEVEL 2	5.03390	0.046651	0.059986
LEVEL 3	5.48649	0.044100	0.058018
LEVEL 4	6.86231	0.034637	0.048873
LEVEL 5	4.10786	0.022878	0.028077

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=48 -----
 (continued)

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN
LEVEL 6	3	.005465922	.009791742	.007422501

TREATMNT	STD_DEV	CV	LOWER_CI	UPPER_CI
LEVEL 6	.002192236	29.5350	.001976685	0.012868

----- T1=0 TN=72 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.050522	0.055657	0.053377	.002235416
LEVEL 1	3	0.052558	0.054609	0.053346	.001104832
LEVEL 2	3	0.051234	0.054334	0.052813	.001550408
LEVEL 3	3	0.048135	0.053474	0.051062	.002706540
LEVEL 4	3	0.045776	0.048135	0.047349	.001362379
LEVEL 5	3	0.028350	0.031557	0.030037	.001610051
LEVEL 6	3	-0.001619	0.002532	0.000305	.002092079

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	4.188	0.051031	0.055722
LEVEL 1	2.071	0.050601	0.056090
LEVEL 2	2.936	0.048961	0.056664
LEVEL 3	5.300	0.044339	0.057786
LEVEL 4	2.877	0.043964	0.050733
LEVEL 5	5.360	0.026038	0.034037
LEVEL 6	686.889	-0.004892	0.005502

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=96 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.051326	0.053800	0.052454	.000952946
LEVEL 1	3	0.051401	0.052536	0.051950	.000568363
LEVEL 2	3	0.051550	0.052735	0.052228	.000611306
LEVEL 3	3	0.050941	0.053187	0.052107	.001125406
LEVEL 4	3	0.047326	0.050295	0.048667	.001505222
LEVEL 5	3	0.031206	0.033530	0.031980	.001342000
LEVEL 6	3	0.000000	0.004896	0.001632	.002826633

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	1.817	0.051454	0.053455
LEVEL 1	1.094	0.050538	0.053362
LEVEL 2	1.170	0.050710	0.053747
LEVEL 3	2.160	0.049312	0.054903
LEVEL 4	3.093	0.044927	0.052406
LEVEL 5	4.196	0.028647	0.035314
LEVEL 6	173.205	-0.005390	0.008654

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA	TRANSFORMED
		p VALUE	p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	2.89	0.0398
TRANSFORMED	6	17	9.82	0.0001

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 11
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 12
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	774.666667	129.111111	5.99
Error	17	366.333333	21.549020	
Corrected Total	23	1141.000000		

Source	Pr > F
Model	0.0016
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.678937	37.13674	4.642092	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	774.666667	129.111111	5.99

Source	Pr > F
TREATMNT	0.0016

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	21.54902
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	1.167	-8.365	10.698	
LEVEL 2 - CONTROL	0.500	-9.031	10.031	
LEVEL 3 - CONTROL	-2.500	-12.031	7.031	
LEVEL 4 - CONTROL	-6.167	-15.698	3.365	
LEVEL 5 - CONTROL	-11.667	-21.198	-2.135	***
LEVEL 6 - CONTROL	-14.667	-24.198	-5.135	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.049	0.003		
LEVEL 1	3	0.050	0.003	0.9990	
LEVEL 2	3	0.049	0.007	1.0000	
LEVEL 3	3	0.047	0.008	0.9527	
LEVEL 4	3	0.044	0.004	0.3222	
LEVEL 5	3	0.019	0.004	0.0130	*
LEVEL 6	3	0.006	0.004	0.0019	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 4.6421.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 48

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA	TRANSFORMED
		p VALUE	p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.68	0.1866
TRANSFORMED	6	17	4.57	0.0062

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 16
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=48 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6
	Number of Observations Read	24
	Number of Observations Used	24

ABC LABORATORIES, INC. 17
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=48 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	926.791667	154.465278	13.49
Error	17	194.708333	11.453431	
Corrected Total	23	1121.500000		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.826386	27.07433	3.384292	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	926.7916667	154.4652778	13.49

Source	Pr > F
TREATMNT	<.0001

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=48 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	11.45343
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-2.250	-9.199	4.699	
LEVEL 1 - CONTROL	-3.917	-10.865	3.032	
LEVEL 3 - CONTROL	-5.083	-12.032	1.865	
LEVEL 4 - CONTROL	-11.250	-18.199	-4.301	***
LEVEL 5 - CONTROL	-14.250	-21.199	-7.301	***
LEVEL 6 - CONTROL	-17.250	-24.199	-10.301	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 48

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.054	0.001		
LEVEL 1	3	0.052	0.001	0.4573	
LEVEL 2	3	0.053	0.003	0.8879	
LEVEL 3	3	0.051	0.003	0.2162	
LEVEL 4	3	0.042	0.003	0.0012	*
LEVEL 5	3	0.025	0.001	0.0001	*
LEVEL 6	3	0.007	0.002	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.3843.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 72

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	<0.01	<0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	0.85	0.5475
TRANSFORMED	6	17	0.84	0.5585

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 21
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=72 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 22
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=72 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	885.500000	147.583333	9.58
Error	17	262.000000	15.411765	
Corrected Total	23	1147.500000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.771678	31.40626	3.925782	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	885.500000	147.5833333	9.58

Source	Pr > F
TREATMNT	0.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=72 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.41176
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	-0.833	-8.894	7.227	
LEVEL 2 - CONTROL	-1.833	-9.894	6.227	
LEVEL 3 - CONTROL	-4.333	-12.394	3.727	
LEVEL 4 - CONTROL	-10.000	-18.061	-1.939	***
LEVEL 5 - CONTROL	-13.333	-21.394	-5.273	***
LEVEL 6 - CONTROL	-16.333	-24.394	-8.273	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 72

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.053	0.002		
LEVEL 1	3	0.053	0.001	0.9996	
LEVEL 2	3	0.053	0.002	0.9753	
LEVEL 3	3	0.051	0.003	0.5048	
LEVEL 4	3	0.047	0.001	0.0118	*
LEVEL 5	3	0.030	0.002	0.0009	*
LEVEL 6	3	0.000	0.002	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9258.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.37	0.0225
TRANSFORMED	6	17	3.67	0.0159

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 26
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
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 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	880.000000	146.666667	9.30
Error	17	268.000000	15.764706	
Corrected Total	23	1148.000000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.766551	31.76383	3.970479	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	880.000000	146.666667	9.30

Source	Pr > F
TREATMNT	0.0001

ABC LABORATORIES, INC. 28
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.76471
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
LEVEL 2 - CONTROL	-0.667	-8.819 7.486	
LEVEL 3 - CONTROL	-1.667	-9.819 6.486	
LEVEL 1 - CONTROL	-2.667	-10.819 5.486	
LEVEL 4 - CONTROL	-10.000	-18.152 -1.848	***
LEVEL 5 - CONTROL	-13.000	-21.152 -4.848	***
LEVEL 6 - CONTROL	-16.000	-24.152 -7.848	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.052	0.001		
LEVEL 1	3	0.052	0.001	0.8835	
LEVEL 2	3	0.052	0.001	0.9999	
LEVEL 3	3	0.052	0.001	0.9852	
LEVEL 4	3	0.049	0.002	0.0128	*
LEVEL 5	3	0.032	0.001	0.0013	*
LEVEL 6	3	0.002	0.003	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9705.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=0 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0	A	1
CONTROL	0	B	1
CONTROL	0	C	1
CONTROL	0	D	1
CONTROL	0	E	1
CONTROL	0	F	1

N = 6

----- HOUR=24 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.00	A	3.1
CONTROL	0.00	B	3.3
CONTROL	0.00	C	3.0
CONTROL	0.00	D	3.2
CONTROL	0.00	E	3.3
CONTROL	0.00	F	3.6
LEVEL 1	1.69	A	3.2
LEVEL 1	1.69	B	3.2
LEVEL 1	1.69	C	3.6
LEVEL 2	3.48	A	2.7
LEVEL 2	3.48	B	3.6
LEVEL 2	3.48	C	3.6
LEVEL 3	7.38	A	3.9
LEVEL 3	7.38	B	2.8
LEVEL 3	7.38	C	2.7
LEVEL 4	15.00	A	2.9
LEVEL 4	15.00	B	2.6
LEVEL 4	15.00	C	3.1
LEVEL 5	28.90	A	1.7
LEVEL 5	28.90	B	1.4
LEVEL 5	28.90	C	1.6
LEVEL 6	44.90	A	1.1
LEVEL 6	44.90	B	1.1
LEVEL 6	44.90	C	1.3

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=48 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.00	A	12.0
CONTROL	0.00	B	13.0
CONTROL	0.00	C	13.0
CONTROL	0.00	D	14.0
CONTROL	0.00	E	13.0
CONTROL	0.00	F	14.0
LEVEL 1	1.69	A	13.0
LEVEL 1	1.69	B	12.0
LEVEL 1	1.69	C	12.0
LEVEL 2	3.48	A	12.0
LEVEL 2	3.48	B	15.0
LEVEL 2	3.48	C	12.0
LEVEL 3	7.38	A	12.0
LEVEL 3	7.38	B	10.0
LEVEL 3	7.38	C	13.0
LEVEL 4	15.00	A	6.4
LEVEL 4	15.00	B	7.6
LEVEL 4	15.00	C	8.4
LEVEL 5	28.90	A	3.3
LEVEL 5	28.90	B	3.6
LEVEL 5	28.90	C	3.3
LEVEL 6	44.90	A	1.6
LEVEL 6	44.90	B	1.4
LEVEL 6	44.90	C	1.3

N = 24

----- HOUR=72 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0	A	39
CONTROL	0	B	38
CONTROL	0	C	52
CONTROL	0	D	55
CONTROL	0	E	46
CONTROL	0	F	53

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=72 -----
 (continued)

TREATMNT	CONC	REP	CELL_104
LEVEL 1	1.69	A	45.00
LEVEL 1	1.69	B	44.00
LEVEL 1	1.69	C	51.00
LEVEL 2	3.48	A	40.00
LEVEL 2	3.48	B	45.00
LEVEL 2	3.48	C	50.00
LEVEL 3	7.38	A	41.00
LEVEL 3	7.38	B	32.00
LEVEL 3	7.38	C	47.00
LEVEL 4	15.00	A	32.00
LEVEL 4	15.00	B	32.00
LEVEL 4	15.00	C	27.00
LEVEL 5	28.90	A	7.70
LEVEL 5	28.90	B	9.70
LEVEL 5	28.90	C	8.80
LEVEL 6	44.90	A	1.00
LEVEL 6	44.90	B	0.89
LEVEL 6	44.90	C	1.20

N = 24

----- HOUR=96 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.00	A	139
CONTROL	0.00	B	138
CONTROL	0.00	C	175
CONTROL	0.00	D	157
CONTROL	0.00	E	155
CONTROL	0.00	F	162
LEVEL 1	1.69	A	139
LEVEL 1	1.69	B	146
LEVEL 1	1.69	C	155
LEVEL 2	3.48	A	141
LEVEL 2	3.48	B	158

ABC LABORATORIES, INC. 33
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=96 -----
(continued)

TREATMNT	CONC	REP	CELL_104
LEVEL 2	3.48	C	153.0
LEVEL 3	7.38	A	165.0
LEVEL 3	7.38	B	133.0
LEVEL 3	7.38	C	150.0
LEVEL 4	15.00	A	125.0
LEVEL 4	15.00	B	104.0
LEVEL 4	15.00	C	94.0
LEVEL 5	28.90	A	20.0
LEVEL 5	28.90	B	20.0
LEVEL 5	28.90	C	25.0
LEVEL 6	44.90	A	1.0
LEVEL 6	44.90	B	1.0
LEVEL 6	44.90	C	1.6

N = 24

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=24 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.047142	0.049041	3.9
CONTROL	0.00	B	0.049747	0.049041	-1.3
CONTROL	0.00	C	0.045776	0.049041	6.7
CONTROL	0.00	D	0.048465	0.049041	1.2
CONTROL	0.00	E	0.049747	0.049041	-1.3
CONTROL	0.00	F	0.053372	0.049041	-8.7
LEVEL 1	1.69	A	0.048465	0.049041	1.2
LEVEL 1	1.69	B	0.048465	0.049041	1.2
LEVEL 1	1.69	C	0.053372	0.049041	-8.7
LEVEL 2	3.48	A	0.041385	0.049041	15.6
LEVEL 2	3.48	B	0.053372	0.049041	-8.7
LEVEL 2	3.48	C	0.053372	0.049041	-8.7
LEVEL 3	7.38	A	0.056707	0.049041	-15.5
LEVEL 3	7.38	B	0.042901	0.049041	12.5
LEVEL 3	7.38	C	0.041385	0.049041	15.6
LEVEL 4	15.00	A	0.044363	0.049041	9.5
LEVEL 4	15.00	B	0.039813	0.049041	18.8
LEVEL 4	15.00	C	0.047142	0.049041	3.9
LEVEL 5	28.90	A	0.022110	0.049041	54.9
LEVEL 5	28.90	B	0.014020	0.049041	71.4
LEVEL 5	28.90	C	0.019583	0.049041	60.1
LEVEL 6	44.90	A	0.003971	0.049041	91.9
LEVEL 6	44.90	B	0.003971	0.049041	91.9
LEVEL 6	44.90	C	0.010932	0.049041	77.7

N = 24

ABC LABORATORIES, INC. 35
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=48 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.051769	0.053673	3.5
CONTROL	0.00	B	0.053436	0.053673	0.4
CONTROL	0.00	C	0.053436	0.053673	0.4
CONTROL	0.00	D	0.054980	0.053673	-2.3
CONTROL	0.00	E	0.053436	0.053673	0.4
CONTROL	0.00	F	0.054980	0.053673	-2.3
LEVEL 1	1.69	A	0.053436	0.053673	0.4
LEVEL 1	1.69	B	0.051769	0.053673	3.5
LEVEL 1	1.69	C	0.051769	0.053673	3.5
LEVEL 2	3.48	A	0.051769	0.053673	3.5
LEVEL 2	3.48	B	0.056418	0.053673	-5.0
LEVEL 2	3.48	C	0.051769	0.053673	3.5
LEVEL 3	7.38	A	0.051769	0.053673	3.5
LEVEL 3	7.38	B	0.047971	0.053673	10.6
LEVEL 3	7.38	C	0.053436	0.053673	0.4
LEVEL 4	15.00	A	0.038673	0.053673	27.9
LEVEL 4	15.00	B	0.042253	0.053673	21.3
LEVEL 4	15.00	C	0.044338	0.053673	17.4
LEVEL 5	28.90	A	0.024873	0.053673	53.7
LEVEL 5	28.90	B	0.026686	0.053673	50.3
LEVEL 5	28.90	C	0.024873	0.053673	53.7
LEVEL 6	44.90	A	0.009792	0.053673	81.8
LEVEL 6	44.90	B	0.007010	0.053673	86.9
LEVEL 6	44.90	C	0.005466	0.053673	89.8

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=72 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.050883	0.053377	4.7
CONTROL	0.00	B	0.050522	0.053377	5.3
CONTROL	0.00	C	0.054878	0.053377	-2.7
CONTROL	0.00	D	0.055657	0.053377	-4.2
CONTROL	0.00	E	0.053176	0.053377	0.4
CONTROL	0.00	F	0.055143	0.053377	-3.2
LEVEL 1	1.69	A	0.052870	0.053377	0.9
LEVEL 1	1.69	B	0.052558	0.053377	1.5
LEVEL 1	1.69	C	0.054609	0.053377	-2.2
LEVEL 2	3.48	A	0.051234	0.053377	4.0
LEVEL 2	3.48	B	0.052870	0.053377	0.9
LEVEL 2	3.48	C	0.054334	0.053377	-1.7
LEVEL 3	7.38	A	0.051577	0.053377	3.4
LEVEL 3	7.38	B	0.048135	0.053377	9.8
LEVEL 3	7.38	C	0.053474	0.053377	-0.1
LEVEL 4	15.00	A	0.048135	0.053377	9.8
LEVEL 4	15.00	B	0.048135	0.053377	9.8
LEVEL 4	15.00	C	0.045776	0.053377	14.2
LEVEL 5	28.90	A	0.028350	0.053377	46.9
LEVEL 5	28.90	B	0.031557	0.053377	40.9
LEVEL 5	28.90	C	0.030205	0.053377	43.4
LEVEL 6	44.90	A	0.000000	0.053377	100.0
LEVEL 6	44.90	B	-0.001619	0.053377	103.0
LEVEL 6	44.90	C	0.002532	0.053377	95.3

N = 24

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=96 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.051401	0.052454	2.0
CONTROL	0.00	B	0.051326	0.052454	2.2
CONTROL	0.00	C	0.053800	0.052454	-2.5
CONTROL	0.00	D	0.052669	0.052454	-0.3
CONTROL	0.00	E	0.052536	0.052454	-0.1
CONTROL	0.00	F	0.052996	0.052454	-0.9
LEVEL 1	1.69	A	0.051401	0.052454	2.0
LEVEL 1	1.69	B	0.051913	0.052454	1.0
LEVEL 1	1.69	C	0.052536	0.052454	-0.1
LEVEL 2	3.48	A	0.051550	0.052454	1.7
LEVEL 2	3.48	B	0.052735	0.052454	-0.4
LEVEL 2	3.48	C	0.052400	0.052454	0.1
LEVEL 3	7.38	A	0.053187	0.052454	-1.3
LEVEL 3	7.38	B	0.050941	0.052454	2.9
LEVEL 3	7.38	C	0.052194	0.052454	0.5
LEVEL 4	15.00	A	0.050295	0.052454	4.1
LEVEL 4	15.00	B	0.048379	0.052454	7.8
LEVEL 4	15.00	C	0.047326	0.052454	9.8
LEVEL 5	28.90	A	0.031206	0.052454	40.5
LEVEL 5	28.90	B	0.031206	0.052454	40.5
LEVEL 5	28.90	C	0.033530	0.052454	36.1
LEVEL 6	44.90	A	0.000000	0.052454	100.0
LEVEL 6	44.90	B	0.000000	0.052454	100.0
LEVEL 6	44.90	C	0.004896	0.052454	90.7

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-8.7	6.7	0.0	5.3	-5.5	5.6
LEVEL 1	1.69	3	-8.7	1.2	-2.1	5.8	-16.4	12.2
LEVEL 2	3.48	3	-8.7	15.6	-0.6	14.1	-35.6	34.4
LEVEL 3	7.38	3	-15.5	15.6	4.2	17.2	-38.5	46.9
LEVEL 4	15.00	3	3.9	18.8	10.7	7.5	-7.9	29.5
LEVEL 5	28.90	3	54.9	71.4	62.1	8.4	41.2	83.1
LEVEL 6	44.90	3	77.7	91.9	87.2	8.2	66.8	107.5

----- T1=0 TN=48 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-2.3	3.5	0.0	2.2	-2.2	2.3
LEVEL 1	1.69	3	0.4	3.5	2.5	1.8	-1.8	7.0
LEVEL 2	3.48	3	-5.0	3.5	0.7	5.0	-11.7	13.1
LEVEL 3	7.38	3	0.4	10.6	4.9	5.2	-8.0	17.8
LEVEL 4	15.00	3	17.4	27.9	22.2	5.3	8.9	35.5
LEVEL 5	28.90	3	50.3	53.7	52.5	1.9	47.7	57.4
LEVEL 6	44.90	3	81.8	89.8	86.2	4.1	76.0	96.3

----- T1=0 TN=72 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-4.2	5.3	0.0	4.2	-4.3	4.4
LEVEL 1	1.69	3	-2.2	1.5	0.1	2.1	-5.0	5.2
LEVEL 2	3.48	3	-1.7	4.0	1.1	2.9	-6.1	8.3
LEVEL 3	7.38	3	-0.1	9.8	4.3	5.1	-8.2	16.9
LEVEL 4	15.00	3	9.8	14.2	11.3	2.6	5.0	17.6
LEVEL 5	28.90	3	40.9	46.9	43.7	3.0	36.2	51.2
LEVEL 6	44.90	3	95.3	103.0	99.4	3.9	89.7	109.2

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=96 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-2.5	2.2	0.0	1.8	-1.8	1.9
LEVEL 1	1.69	3	-0.1	2.0	1.0	1.1	-1.6	3.7
LEVEL 2	3.48	3	-0.4	1.7	0.4	1.2	-2.4	3.3
LEVEL 3	7.38	3	-1.3	2.9	0.7	2.1	-4.6	6.0
LEVEL 4	15.00	3	4.1	9.8	7.2	2.9	0.1	14.3
LEVEL 5	28.90	3	36.1	40.5	39.0	2.6	32.7	45.4
LEVEL 6	44.90	3	90.7	100.0	96.9	5.4	83.5	110.3

ABC LABORATORIES, INC. 40
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.48 AND EC50= 25.337247508

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 228.07 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 9.75
 R-SQUARED= 93.40

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 10.873557712 95% LOWER LIMIT= 7.7678665367 95% UPPER LIMIT= 13.979248888
PERCENT= 10	EC= 13.477339625 95% LOWER LIMIT= 10.377287464 95% UPPER LIMIT= 16.577391785
PERCENT= 20	EC= 17.01322801 95% LOWER LIMIT= 14.104012766 95% UPPER LIMIT= 19.922443254
PERCENT= 50	EC= 25.337247508 95% LOWER LIMIT= 23.259519211 95% UPPER LIMIT= 27.414975805
PERCENT= 90	EC= 47.633741462 , GREATER THAN HIGHEST CONC.= 44.9 95% LOWER LIMIT= 41.345036032 95% UPPER LIMIT= 53.922446891

ABC LABORATORIES, INC. 41
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 48

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.40 AND EC50= 27.293717811

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 438.32 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 6.31
 R-SQUARED= 96.50

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 11.487036209 95% LOWER LIMIT= 9.4228999648 95% UPPER LIMIT= 13.551172454
PERCENT= 10	EC= 14.308337159 95% LOWER LIMIT= 12.252352224 95% UPPER LIMIT= 16.364322094
PERCENT= 20	EC= 18.159488049 95% LOWER LIMIT= 16.243253263 95% UPPER LIMIT= 20.075722834
PERCENT= 50	EC= 27.293717811 95% LOWER LIMIT= 25.956119143 95% UPPER LIMIT= 28.63131648
PERCENT= 90	EC= 52.063843876 , GREATER THAN HIGHEST CONC.= 44.9 95% LOWER LIMIT= 47.401467098 95% UPPER LIMIT= 56.726220654

ABC LABORATORIES, INC. 42
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 72

ESTIMATES OF COEFFICIENTS:
 SLOPE= 10.00 AND EC50= 29.622381268

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 627.59 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 6.00
 R-SQUARED= 97.50

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 22.067066674 95% LOWER LIMIT= 21.809527579 95% UPPER LIMIT= 22.324605769
PERCENT= 10	EC= 23.779116597 95% LOWER LIMIT= 23.501596605 95% UPPER LIMIT= 24.05663659
PERCENT= 20	EC= 25.787780696 95% LOWER LIMIT= 25.486818098 95% UPPER LIMIT= 26.088743294
PERCENT= 50	EC= 29.622381268 95% LOWER LIMIT= 29.276666027 95% UPPER LIMIT= 29.968096509
PERCENT= 90	EC= 36.901516859 95% LOWER LIMIT= 36.470848687 95% UPPER LIMIT= 37.332185031

ABC LABORATORIES, INC. 43
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

ESTIMATES OF COEFFICIENTS:
 SLOPE= 8.82 AND EC50= 30.399520504

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 1395.33 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 3.99
 R-SQUARED= 98.90

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 21.767448664 95% LOWER LIMIT= 20.323684983 95% UPPER LIMIT= 23.211212345
PERCENT= 10	EC= 23.692964642 95% LOWER LIMIT= 22.526663123 95% UPPER LIMIT= 24.859266161
PERCENT= 20	EC= 25.975880281 95% LOWER LIMIT= 25.148969693 95% UPPER LIMIT= 26.802790869
PERCENT= 50	EC= 30.399520504 95% LOWER LIMIT= 29.81105327 95% UPPER LIMIT= 30.987987737
PERCENT= 90	EC= 39.004441226 95% LOWER LIMIT= 36.555733 95% UPPER LIMIT= 41.453149453

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK ON: 14JUL10
14 JUL 10

THE ANALYSIS WAS REVIEWED

BY: AAL ON: 20SEP10

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	3.1
24	CONTROL	B	3.3
24	CONTROL	C	3.0
24	CONTROL	D	3.2
24	CONTROL	E	3.3
24	CONTROL	F	3.6
24	LEVEL 1	A	3.2
24	LEVEL 1	B	3.2
24	LEVEL 1	C	3.6
24	LEVEL 2	A	2.7
24	LEVEL 2	B	3.6
24	LEVEL 2	C	3.6
24	LEVEL 3	A	3.9
24	LEVEL 3	B	2.8
24	LEVEL 3	C	2.7
24	LEVEL 4	A	2.9
24	LEVEL 4	B	2.6
24	LEVEL 4	C	3.1
24	LEVEL 5	A	1.7
24	LEVEL 5	B	1.4
24	LEVEL 5	C	1.6
24	LEVEL 6	A	1.1
24	LEVEL 6	B	1.1
24	LEVEL 6	C	1.3
48	CONTROL	A	12.0
48	CONTROL	B	13.0
48	CONTROL	C	13.0
48	CONTROL	D	14.0
48	CONTROL	E	13.0
48	CONTROL	F	14.0
48	LEVEL 1	A	13.0
48	LEVEL 1	B	12.0
48	LEVEL 1	C	12.0
48	LEVEL 2	A	12.0
48	LEVEL 2	B	15.0
48	LEVEL 2	C	12.0
48	LEVEL 3	A	12.0
48	LEVEL 3	B	10.0

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
48	LEVEL 3	C	13.00
48	LEVEL 4	A	6.40
48	LEVEL 4	B	7.60
48	LEVEL 4	C	8.40
48	LEVEL 5	A	3.30
48	LEVEL 5	B	3.60
48	LEVEL 5	C	3.30
48	LEVEL 6	A	1.60
48	LEVEL 6	B	1.40
48	LEVEL 6	C	1.30
72	CONTROL	A	39.00
72	CONTROL	B	38.00
72	CONTROL	C	52.00
72	CONTROL	D	55.00
72	CONTROL	E	46.00
72	CONTROL	F	53.00
72	LEVEL 1	A	45.00
72	LEVEL 1	B	44.00
72	LEVEL 1	C	51.00
72	LEVEL 2	A	40.00
72	LEVEL 2	B	45.00
72	LEVEL 2	C	50.00
72	LEVEL 3	A	41.00
72	LEVEL 3	B	32.00
72	LEVEL 3	C	47.00
72	LEVEL 4	A	32.00
72	LEVEL 4	B	32.00
72	LEVEL 4	C	27.00
72	LEVEL 5	A	7.70
72	LEVEL 5	B	9.70
72	LEVEL 5	C	8.80
72	LEVEL 6	A	1.00
72	LEVEL 6	B	0.89
72	LEVEL 6	C	1.20
96	CONTROL	A	139.00
96	CONTROL	B	138.00
96	CONTROL	C	175.00
96	CONTROL	D	157.00
96	CONTROL	E	155.00
96	CONTROL	F	162.00
96	LEVEL 1	A	139.00
96	LEVEL 1	B	146.00
96	LEVEL 1	C	155.00
96	LEVEL 2	A	141.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
96	LEVEL 2	B	158.0
96	LEVEL 2	C	153.0
96	LEVEL 3	A	165.0
96	LEVEL 3	B	133.0
96	LEVEL 3	C	150.0
96	LEVEL 4	A	125.0
96	LEVEL 4	B	104.0
96	LEVEL 4	C	94.0
96	LEVEL 5	A	20.0
96	LEVEL 5	B	20.0
96	LEVEL 5	C	25.0
96	LEVEL 6	A	1.0
96	LEVEL 6	B	1.0
96	LEVEL 6	C	1.6

N = 102

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	24	CONTROL	A	0.047142
0	24	CONTROL	B	0.049747
0	24	CONTROL	C	0.045776
0	24	CONTROL	D	0.048465
0	24	CONTROL	E	0.049747
0	24	CONTROL	F	0.053372
0	24	LEVEL 1	A	0.048465
0	24	LEVEL 1	B	0.048465
0	24	LEVEL 1	C	0.053372
0	24	LEVEL 2	A	0.041385
0	24	LEVEL 2	B	0.053372
0	24	LEVEL 2	C	0.053372
0	24	LEVEL 3	A	0.056707
0	24	LEVEL 3	B	0.042901
0	24	LEVEL 3	C	0.041385
0	24	LEVEL 4	A	0.044363
0	24	LEVEL 4	B	0.039813
0	24	LEVEL 4	C	0.047142
0	24	LEVEL 5	A	0.022110
0	24	LEVEL 5	B	0.014020
0	24	LEVEL 5	C	0.019583
0	24	LEVEL 6	A	0.003971
0	24	LEVEL 6	B	0.003971
0	24	LEVEL 6	C	0.010932
0	48	CONTROL	A	0.051769
0	48	CONTROL	B	0.053436
0	48	CONTROL	C	0.053436
0	48	CONTROL	D	0.054980
0	48	CONTROL	E	0.053436
0	48	CONTROL	F	0.054980
0	48	LEVEL 1	A	0.053436
0	48	LEVEL 1	B	0.051769
0	48	LEVEL 1	C	0.051769
0	48	LEVEL 2	A	0.051769
0	48	LEVEL 2	B	0.056418
0	48	LEVEL 2	C	0.051769
0	48	LEVEL 3	A	0.051769
0	48	LEVEL 3	B	0.047971
0	48	LEVEL 3	C	0.053436
0	48	LEVEL 4	A	0.038673
0	48	LEVEL 4	B	0.042253
0	48	LEVEL 4	C	0.044338
0	48	LEVEL 5	A	0.024873
0	48	LEVEL 5	B	0.026686

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	48	LEVEL 5	C	0.024873
0	48	LEVEL 6	A	0.009792
0	48	LEVEL 6	B	0.007010
0	48	LEVEL 6	C	0.005466
0	72	CONTROL	A	0.050883
0	72	CONTROL	B	0.050522
0	72	CONTROL	C	0.054878
0	72	CONTROL	D	0.055657
0	72	CONTROL	E	0.053176
0	72	CONTROL	F	0.055143
0	72	LEVEL 1	A	0.052870
0	72	LEVEL 1	B	0.052558
0	72	LEVEL 1	C	0.054609
0	72	LEVEL 2	A	0.051234
0	72	LEVEL 2	B	0.052870
0	72	LEVEL 2	C	0.054334
0	72	LEVEL 3	A	0.051577
0	72	LEVEL 3	B	0.048135
0	72	LEVEL 3	C	0.053474
0	72	LEVEL 4	A	0.048135
0	72	LEVEL 4	B	0.048135
0	72	LEVEL 4	C	0.045776
0	72	LEVEL 5	A	0.028350
0	72	LEVEL 5	B	0.031557
0	72	LEVEL 5	C	0.030205
0	72	LEVEL 6	A	0.000000
0	72	LEVEL 6	B	-0.001619
0	72	LEVEL 6	C	0.002532
0	96	CONTROL	A	0.051401
0	96	CONTROL	B	0.051326
0	96	CONTROL	C	0.053800
0	96	CONTROL	D	0.052669
0	96	CONTROL	E	0.052536
0	96	CONTROL	F	0.052996
0	96	LEVEL 1	A	0.051401
0	96	LEVEL 1	B	0.051913
0	96	LEVEL 1	C	0.052536
0	96	LEVEL 2	A	0.051550
0	96	LEVEL 2	B	0.052735
0	96	LEVEL 2	C	0.052400
0	96	LEVEL 3	A	0.053187
0	96	LEVEL 3	B	0.050941
0	96	LEVEL 3	C	0.052194
0	96	LEVEL 4	A	0.050295

ABC LABORATORIES, INC. 6
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	96	LEVEL 4	B	0.048379
0	96	LEVEL 4	C	0.047326
0	96	LEVEL 5	A	0.031206
0	96	LEVEL 5	B	0.031206
0	96	LEVEL 5	C	0.033530
0	96	LEVEL 6	A	0.000000
0	96	LEVEL 6	B	0.000000
0	96	LEVEL 6	C	0.004896

N = 96

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.045776	0.053372	0.049041	.002621399
LEVEL 1	3	0.048465	0.053372	0.050100	.002833419
LEVEL 2	3	0.041385	0.053372	0.049377	.006920555
LEVEL 3	3	0.041385	0.056707	0.046998	.008442714
LEVEL 4	3	0.039813	0.047142	0.043773	.003699887
LEVEL 5	3	0.014020	0.022110	0.018571	.004138884
LEVEL 6	3	0.003971	0.010932	0.006291	.004018697

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	5.3453	0.046290	0.051792
LEVEL 1	5.6555	0.043062	0.057139
LEVEL 2	14.0158	0.032185	0.066568
LEVEL 3	17.9640	0.026025	0.067971
LEVEL 4	8.4525	0.034582	0.052964
LEVEL 5	22.2869	0.008289	0.028852
LEVEL 6	63.8755	-0.003692	0.016274

----- T1=0 TN=48 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.051769	0.054980	0.053673	.001200991
LEVEL 1	3	0.051769	0.053436	0.052325	.000962764
LEVEL 2	3	0.051769	0.056418	0.053318	.002684000
LEVEL 3	3	0.047971	0.053436	0.051059	.002801328
LEVEL 4	3	0.038673	0.044338	0.041755	.002865336
LEVEL 5	3	0.024873	0.026686	0.025478	.001046584

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	2.23760	0.052413	0.054934
LEVEL 1	1.83998	0.049933	0.054716
LEVEL 2	5.03390	0.046651	0.059986
LEVEL 3	5.48649	0.044100	0.058018
LEVEL 4	6.86231	0.034637	0.048873
LEVEL 5	4.10786	0.022878	0.028077

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=48 -----
 (continued)

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN
LEVEL 6	3	.005465922	.009791742	.007422501

TREATMNT	STD_DEV	CV	LOWER_CI	UPPER_CI
LEVEL 6	.002192236	29.5350	.001976685	0.012868

----- T1=0 TN=72 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.050522	0.055657	0.053377	.002235416
LEVEL 1	3	0.052558	0.054609	0.053346	.001104832
LEVEL 2	3	0.051234	0.054334	0.052813	.001550408
LEVEL 3	3	0.048135	0.053474	0.051062	.002706540
LEVEL 4	3	0.045776	0.048135	0.047349	.001362379
LEVEL 5	3	0.028350	0.031557	0.030037	.001610051
LEVEL 6	3	-0.001619	0.002532	0.000305	.002092079

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	4.188	0.051031	0.055722
LEVEL 1	2.071	0.050601	0.056090
LEVEL 2	2.936	0.048961	0.056664
LEVEL 3	5.300	0.044339	0.057786
LEVEL 4	2.877	0.043964	0.050733
LEVEL 5	5.360	0.026038	0.034037
LEVEL 6	686.889	-0.004892	0.005502

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=96 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.051326	0.053800	0.052454	.000952946
LEVEL 1	3	0.051401	0.052536	0.051950	.000568363
LEVEL 2	3	0.051550	0.052735	0.052228	.000611306
LEVEL 3	3	0.050941	0.053187	0.052107	.001125406
LEVEL 4	3	0.047326	0.050295	0.048667	.001505222
LEVEL 5	3	0.031206	0.033530	0.031980	.001342000
LEVEL 6	3	0.000000	0.004896	0.001632	.002826633

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	1.817	0.051454	0.053455
LEVEL 1	1.094	0.050538	0.053362
LEVEL 2	1.170	0.050710	0.053747
LEVEL 3	2.160	0.049312	0.054903
LEVEL 4	3.093	0.044927	0.052406
LEVEL 5	4.196	0.028647	0.035314
LEVEL 6	173.205	-0.005390	0.008654

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA	TRANSFORMED
		p VALUE	p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.37	0.0225
TRANSFORMED	6	17	3.67	0.0159

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 11
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 12
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	880.000000	146.666667	9.30
Error	17	268.000000	15.764706	
Corrected Total	23	1148.000000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.766551	31.76383	3.970479	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	880.000000	146.666667	9.30

Source	Pr > F
TREATMNT	0.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=96 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.76471
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-0.667	-8.819	7.486	
LEVEL 3 - CONTROL	-1.667	-9.819	6.486	
LEVEL 1 - CONTROL	-2.667	-10.819	5.486	
LEVEL 4 - CONTROL	-10.000	-18.152	-1.848	***
LEVEL 5 - CONTROL	-13.000	-21.152	-4.848	***
LEVEL 6 - CONTROL	-16.000	-24.152	-7.848	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.052	0.001		
LEVEL 1	3	0.052	0.001	0.8835	
LEVEL 2	3	0.052	0.001	0.9999	
LEVEL 3	3	0.052	0.001	0.9852	
LEVEL 4	3	0.049	0.002	0.0128	*
LEVEL 5	3	0.032	0.001	0.0013	*
LEVEL 6	3	0.002	0.003	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9705.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=0 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0	A	1
CONTROL	0	B	1
CONTROL	0	C	1
CONTROL	0	D	1
CONTROL	0	E	1
CONTROL	0	F	1

N = 6

----- HOUR=24 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.00	A	3.1
CONTROL	0.00	B	3.3
CONTROL	0.00	C	3.0
CONTROL	0.00	D	3.2
CONTROL	0.00	E	3.3
CONTROL	0.00	F	3.6
LEVEL 1	1.64	A	3.2
LEVEL 1	1.64	B	3.2
LEVEL 1	1.64	C	3.6
LEVEL 2	3.51	A	2.7
LEVEL 2	3.51	B	3.6
LEVEL 2	3.51	C	3.6
LEVEL 3	7.41	A	3.9
LEVEL 3	7.41	B	2.8
LEVEL 3	7.41	C	2.7
LEVEL 4	14.80	A	2.9
LEVEL 4	14.80	B	2.6
LEVEL 4	14.80	C	3.1
LEVEL 5	28.40	A	1.7
LEVEL 5	28.40	B	1.4
LEVEL 5	28.40	C	1.6
LEVEL 6	44.80	A	1.1
LEVEL 6	44.80	B	1.1
LEVEL 6	44.80	C	1.3

N = 24

ABC LABORATORIES, INC. 16
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=48 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.00	A	12.0
CONTROL	0.00	B	13.0
CONTROL	0.00	C	13.0
CONTROL	0.00	D	14.0
CONTROL	0.00	E	13.0
CONTROL	0.00	F	14.0
LEVEL 1	1.64	A	13.0
LEVEL 1	1.64	B	12.0
LEVEL 1	1.64	C	12.0
LEVEL 2	3.51	A	12.0
LEVEL 2	3.51	B	15.0
LEVEL 2	3.51	C	12.0
LEVEL 3	7.41	A	12.0
LEVEL 3	7.41	B	10.0
LEVEL 3	7.41	C	13.0
LEVEL 4	14.80	A	6.4
LEVEL 4	14.80	B	7.6
LEVEL 4	14.80	C	8.4
LEVEL 5	28.40	A	3.3
LEVEL 5	28.40	B	3.6
LEVEL 5	28.40	C	3.3
LEVEL 6	44.80	A	1.6
LEVEL 6	44.80	B	1.4
LEVEL 6	44.80	C	1.3

N = 24

----- HOUR=72 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0	A	39
CONTROL	0	B	38
CONTROL	0	C	52
CONTROL	0	D	55
CONTROL	0	E	46
CONTROL	0	F	53

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=72 -----
 (continued)

TREATMNT	CONC	REP	CELL_104
LEVEL 1	1.64	A	45.00
LEVEL 1	1.64	B	44.00
LEVEL 1	1.64	C	51.00
LEVEL 2	3.51	A	40.00
LEVEL 2	3.51	B	45.00
LEVEL 2	3.51	C	50.00
LEVEL 3	7.41	A	41.00
LEVEL 3	7.41	B	32.00
LEVEL 3	7.41	C	47.00
LEVEL 4	14.80	A	32.00
LEVEL 4	14.80	B	32.00
LEVEL 4	14.80	C	27.00
LEVEL 5	28.40	A	7.70
LEVEL 5	28.40	B	9.70
LEVEL 5	28.40	C	8.80
LEVEL 6	44.80	A	1.00
LEVEL 6	44.80	B	0.89
LEVEL 6	44.80	C	1.20

N = 24

----- HOUR=96 -----

TREATMNT	CONC	REP	CELL_104
CONTROL	0.00	A	139
CONTROL	0.00	B	138
CONTROL	0.00	C	175
CONTROL	0.00	D	157
CONTROL	0.00	E	155
CONTROL	0.00	F	162
LEVEL 1	1.64	A	139
LEVEL 1	1.64	B	146
LEVEL 1	1.64	C	155
LEVEL 2	3.51	A	141
LEVEL 2	3.51	B	158

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

----- HOUR=96 -----
 (continued)

TREATMNT	CONC	REP	CELL_104
LEVEL 2	3.51	C	153.0
LEVEL 3	7.41	A	165.0
LEVEL 3	7.41	B	133.0
LEVEL 3	7.41	C	150.0
LEVEL 4	14.80	A	125.0
LEVEL 4	14.80	B	104.0
LEVEL 4	14.80	C	94.0
LEVEL 5	28.40	A	20.0
LEVEL 5	28.40	B	20.0
LEVEL 5	28.40	C	25.0
LEVEL 6	44.80	A	1.0
LEVEL 6	44.80	B	1.0
LEVEL 6	44.80	C	1.6

N = 24

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=24 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.047142	0.049041	3.9
CONTROL	0.00	B	0.049747	0.049041	-1.3
CONTROL	0.00	C	0.045776	0.049041	6.7
CONTROL	0.00	D	0.048465	0.049041	1.2
CONTROL	0.00	E	0.049747	0.049041	-1.3
CONTROL	0.00	F	0.053372	0.049041	-8.7
LEVEL 1	1.64	A	0.048465	0.049041	1.2
LEVEL 1	1.64	B	0.048465	0.049041	1.2
LEVEL 1	1.64	C	0.053372	0.049041	-8.7
LEVEL 2	3.51	A	0.041385	0.049041	15.6
LEVEL 2	3.51	B	0.053372	0.049041	-8.7
LEVEL 2	3.51	C	0.053372	0.049041	-8.7
LEVEL 3	7.41	A	0.056707	0.049041	-15.5
LEVEL 3	7.41	B	0.042901	0.049041	12.5
LEVEL 3	7.41	C	0.041385	0.049041	15.6
LEVEL 4	14.80	A	0.044363	0.049041	9.5
LEVEL 4	14.80	B	0.039813	0.049041	18.8
LEVEL 4	14.80	C	0.047142	0.049041	3.9
LEVEL 5	28.40	A	0.022110	0.049041	54.9
LEVEL 5	28.40	B	0.014020	0.049041	71.4
LEVEL 5	28.40	C	0.019583	0.049041	60.1
LEVEL 6	44.80	A	0.003971	0.049041	91.9
LEVEL 6	44.80	B	0.003971	0.049041	91.9
LEVEL 6	44.80	C	0.010932	0.049041	77.7

N = 24

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=48 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.051769	0.053673	3.5
CONTROL	0.00	B	0.053436	0.053673	0.4
CONTROL	0.00	C	0.053436	0.053673	0.4
CONTROL	0.00	D	0.054980	0.053673	-2.3
CONTROL	0.00	E	0.053436	0.053673	0.4
CONTROL	0.00	F	0.054980	0.053673	-2.3
LEVEL 1	1.64	A	0.053436	0.053673	0.4
LEVEL 1	1.64	B	0.051769	0.053673	3.5
LEVEL 1	1.64	C	0.051769	0.053673	3.5
LEVEL 2	3.51	A	0.051769	0.053673	3.5
LEVEL 2	3.51	B	0.056418	0.053673	-5.0
LEVEL 2	3.51	C	0.051769	0.053673	3.5
LEVEL 3	7.41	A	0.051769	0.053673	3.5
LEVEL 3	7.41	B	0.047971	0.053673	10.6
LEVEL 3	7.41	C	0.053436	0.053673	0.4
LEVEL 4	14.80	A	0.038673	0.053673	27.9
LEVEL 4	14.80	B	0.042253	0.053673	21.3
LEVEL 4	14.80	C	0.044338	0.053673	17.4
LEVEL 5	28.40	A	0.024873	0.053673	53.7
LEVEL 5	28.40	B	0.026686	0.053673	50.3
LEVEL 5	28.40	C	0.024873	0.053673	53.7
LEVEL 6	44.80	A	0.009792	0.053673	81.8
LEVEL 6	44.80	B	0.007010	0.053673	86.9
LEVEL 6	44.80	C	0.005466	0.053673	89.8

N = 24

ABC LABORATORIES, INC. 21
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=72 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.050883	0.053377	4.7
CONTROL	0.00	B	0.050522	0.053377	5.3
CONTROL	0.00	C	0.054878	0.053377	-2.7
CONTROL	0.00	D	0.055657	0.053377	-4.2
CONTROL	0.00	E	0.053176	0.053377	0.4
CONTROL	0.00	F	0.055143	0.053377	-3.2
LEVEL 1	1.64	A	0.052870	0.053377	0.9
LEVEL 1	1.64	B	0.052558	0.053377	1.5
LEVEL 1	1.64	C	0.054609	0.053377	-2.2
LEVEL 2	3.51	A	0.051234	0.053377	4.0
LEVEL 2	3.51	B	0.052870	0.053377	0.9
LEVEL 2	3.51	C	0.054334	0.053377	-1.7
LEVEL 3	7.41	A	0.051577	0.053377	3.4
LEVEL 3	7.41	B	0.048135	0.053377	9.8
LEVEL 3	7.41	C	0.053474	0.053377	-0.1
LEVEL 4	14.80	A	0.048135	0.053377	9.8
LEVEL 4	14.80	B	0.048135	0.053377	9.8
LEVEL 4	14.80	C	0.045776	0.053377	14.2
LEVEL 5	28.40	A	0.028350	0.053377	46.9
LEVEL 5	28.40	B	0.031557	0.053377	40.9
LEVEL 5	28.40	C	0.030205	0.053377	43.4
LEVEL 6	44.80	A	0.000000	0.053377	100.0
LEVEL 6	44.80	B	-0.001619	0.053377	103.0
LEVEL 6	44.80	C	0.002532	0.053377	95.3

N = 24

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- T1=0 TN=96 -----

TREATMNT	CONC	REP	RATE	CON_MEAN	PER_INH
CONTROL	0.00	A	0.051401	0.052454	2.0
CONTROL	0.00	B	0.051326	0.052454	2.2
CONTROL	0.00	C	0.053800	0.052454	-2.5
CONTROL	0.00	D	0.052669	0.052454	-0.3
CONTROL	0.00	E	0.052536	0.052454	-0.1
CONTROL	0.00	F	0.052996	0.052454	-0.9
LEVEL 1	1.64	A	0.051401	0.052454	2.0
LEVEL 1	1.64	B	0.051913	0.052454	1.0
LEVEL 1	1.64	C	0.052536	0.052454	-0.1
LEVEL 2	3.51	A	0.051550	0.052454	1.7
LEVEL 2	3.51	B	0.052735	0.052454	-0.4
LEVEL 2	3.51	C	0.052400	0.052454	0.1
LEVEL 3	7.41	A	0.053187	0.052454	-1.3
LEVEL 3	7.41	B	0.050941	0.052454	2.9
LEVEL 3	7.41	C	0.052194	0.052454	0.5
LEVEL 4	14.80	A	0.050295	0.052454	4.1
LEVEL 4	14.80	B	0.048379	0.052454	7.8
LEVEL 4	14.80	C	0.047326	0.052454	9.8
LEVEL 5	28.40	A	0.031206	0.052454	40.5
LEVEL 5	28.40	B	0.031206	0.052454	40.5
LEVEL 5	28.40	C	0.033530	0.052454	36.1
LEVEL 6	44.80	A	0.000000	0.052454	100.0
LEVEL 6	44.80	B	0.000000	0.052454	100.0
LEVEL 6	44.80	C	0.004896	0.052454	90.7

N = 24

23

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-8.7	6.7	0.0	5.3	-5.5	5.6
LEVEL 1	1.64	3	-8.7	1.2	-2.1	5.8	-16.4	12.2
LEVEL 2	3.51	3	-8.7	15.6	-0.6	14.1	-35.6	34.4
LEVEL 3	7.41	3	-15.5	15.6	4.2	17.2	-38.5	46.9
LEVEL 4	14.80	3	3.9	18.8	10.7	7.5	-7.9	29.5
LEVEL 5	28.40	3	54.9	71.4	62.1	8.4	41.2	83.1
LEVEL 6	44.80	3	77.7	91.9	87.2	8.2	66.8	107.5

----- T1=0 TN=48 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-2.3	3.5	0.0	2.2	-2.2	2.3
LEVEL 1	1.64	3	0.4	3.5	2.5	1.8	-1.8	7.0
LEVEL 2	3.51	3	-5.0	3.5	0.7	5.0	-11.7	13.1
LEVEL 3	7.41	3	0.4	10.6	4.9	5.2	-8.0	17.8
LEVEL 4	14.80	3	17.4	27.9	22.2	5.3	8.9	35.5
LEVEL 5	28.40	3	50.3	53.7	52.5	1.9	47.7	57.4
LEVEL 6	44.80	3	81.8	89.8	86.2	4.1	76.0	96.3

----- T1=0 TN=72 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-4.2	5.3	0.0	4.2	-4.3	4.4
LEVEL 1	1.64	3	-2.2	1.5	0.1	2.1	-5.0	5.2
LEVEL 2	3.51	3	-1.7	4.0	1.1	2.9	-6.1	8.3
LEVEL 3	7.41	3	-0.1	9.8	4.3	5.1	-8.2	16.9
LEVEL 4	14.80	3	9.8	14.2	11.3	2.6	5.0	17.6
LEVEL 5	28.40	3	40.9	46.9	43.7	3.0	36.2	51.2
LEVEL 6	44.80	3	95.3	103.0	99.4	3.9	89.7	109.2

24

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE FROM TIME ZERO
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=96 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-2.5	2.2	0.0	1.8	-1.8	1.9
LEVEL 1	1.64	3	-0.1	2.0	1.0	1.1	-1.6	3.7
LEVEL 2	3.51	3	-0.4	1.7	0.4	1.2	-2.4	3.3
LEVEL 3	7.41	3	-1.3	2.9	0.7	2.1	-4.6	6.0
LEVEL 4	14.80	3	4.1	9.8	7.2	2.9	0.1	14.3
LEVEL 5	28.40	3	36.1	40.5	39.0	2.6	32.7	45.4
LEVEL 6	44.80	3	90.7	100.0	96.9	5.4	83.5	110.3

ABC LABORATORIES, INC. 25
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE FROM TIME ZERO
ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
RESULTS FROM FITTING THE LOGISTIC MODEL
USING NONLINEAR (WEIGHTED) REGRESSION

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 96

ESTIMATES OF COEFFICIENTS:
SLOPE= 8.52 AND EC50= 29.925877168

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
F= 1407.15 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
ROOT MEAN SQUARE ERROR (RMSE)= 3.97
R-SQUARED= 98.90

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5 EC= 21.181965608
95% LOWER LIMIT= 19.727664551
95% UPPER LIMIT= 22.636266664

PERCENT= 10 EC= 23.123439313
95% LOWER LIMIT= 21.945155911
95% UPPER LIMIT= 24.301722715

PERCENT= 20 EC= 25.432336007
95% LOWER LIMIT= 24.5942359
95% UPPER LIMIT= 26.270436113

PERCENT= 50 EC= 29.925877168
95% LOWER LIMIT= 29.326189046
95% UPPER LIMIT= 30.52556529

PERCENT= 90 EC= 38.729451624
95% LOWER LIMIT= 36.21267618
95% UPPER LIMIT= 41.246227069

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK ON: 14JUL10
MAL 14JUL10

THE ANALYSIS WAS REVIEWED

BY: AAL ON: 20SEP10

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	3.1
24	CONTROL	B	3.3
24	CONTROL	C	3.0
24	CONTROL	D	3.2
24	CONTROL	E	3.3
24	CONTROL	F	3.6
24	LEVEL 1	A	3.2
24	LEVEL 1	B	3.2
24	LEVEL 1	C	3.6
24	LEVEL 2	A	2.7
24	LEVEL 2	B	3.6
24	LEVEL 2	C	3.6
24	LEVEL 3	A	3.9
24	LEVEL 3	B	2.8
24	LEVEL 3	C	2.7
24	LEVEL 4	A	2.9
24	LEVEL 4	B	2.6
24	LEVEL 4	C	3.1
24	LEVEL 5	A	1.7
24	LEVEL 5	B	1.4
24	LEVEL 5	C	1.6
24	LEVEL 6	A	1.1
24	LEVEL 6	B	1.1
24	LEVEL 6	C	1.3
48	CONTROL	A	12.0
48	CONTROL	B	13.0
48	CONTROL	C	13.0
48	CONTROL	D	14.0
48	CONTROL	E	13.0
48	CONTROL	F	14.0
48	LEVEL 1	A	13.0
48	LEVEL 1	B	12.0
48	LEVEL 1	C	12.0
48	LEVEL 2	A	12.0
48	LEVEL 2	B	15.0
48	LEVEL 2	C	12.0
48	LEVEL 3	A	12.0
48	LEVEL 3	B	10.0

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ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
48	LEVEL 3	C	13.00
48	LEVEL 4	A	6.40
48	LEVEL 4	B	7.60
48	LEVEL 4	C	8.40
48	LEVEL 5	A	3.30
48	LEVEL 5	B	3.60
48	LEVEL 5	C	3.30
48	LEVEL 6	A	1.60
48	LEVEL 6	B	1.40
48	LEVEL 6	C	1.30
72	CONTROL	A	39.00
72	CONTROL	B	38.00
72	CONTROL	C	52.00
72	CONTROL	D	55.00
72	CONTROL	E	46.00
72	CONTROL	F	53.00
72	LEVEL 1	A	45.00
72	LEVEL 1	B	44.00
72	LEVEL 1	C	51.00
72	LEVEL 2	A	40.00
72	LEVEL 2	B	45.00
72	LEVEL 2	C	50.00
72	LEVEL 3	A	41.00
72	LEVEL 3	B	32.00
72	LEVEL 3	C	47.00
72	LEVEL 4	A	32.00
72	LEVEL 4	B	32.00
72	LEVEL 4	C	27.00
72	LEVEL 5	A	7.70
72	LEVEL 5	B	9.70
72	LEVEL 5	C	8.80
72	LEVEL 6	A	1.00
72	LEVEL 6	B	0.89
72	LEVEL 6	C	1.20
96	CONTROL	A	139.00
96	CONTROL	B	138.00
96	CONTROL	C	175.00
96	CONTROL	D	157.00
96	CONTROL	E	155.00
96	CONTROL	F	162.00
96	LEVEL 1	A	139.00
96	LEVEL 1	B	146.00
96	LEVEL 1	C	155.00
96	LEVEL 2	A	141.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
PRINTOUT OF NUMBER OF CELLS DIVIDED BY 10**4

HOUR	TREATMNT	REP	CELL_104
96	LEVEL 2	B	158.0
96	LEVEL 2	C	153.0
96	LEVEL 3	A	165.0
96	LEVEL 3	B	133.0
96	LEVEL 3	C	150.0
96	LEVEL 4	A	125.0
96	LEVEL 4	B	104.0
96	LEVEL 4	C	94.0
96	LEVEL 5	A	20.0
96	LEVEL 5	B	20.0
96	LEVEL 5	C	25.0
96	LEVEL 6	A	1.0
96	LEVEL 6	B	1.0
96	LEVEL 6	C	1.6

N = 102

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
0	24	CONTROL	A	0.047142
0	24	CONTROL	B	0.049747
0	24	CONTROL	C	0.045776
0	24	CONTROL	D	0.048465
0	24	CONTROL	E	0.049747
0	24	CONTROL	F	0.053372
0	24	LEVEL 1	A	0.048465
0	24	LEVEL 1	B	0.048465
0	24	LEVEL 1	C	0.053372
0	24	LEVEL 2	A	0.041385
0	24	LEVEL 2	B	0.053372
0	24	LEVEL 2	C	0.053372
0	24	LEVEL 3	A	0.056707
0	24	LEVEL 3	B	0.042901
0	24	LEVEL 3	C	0.041385
0	24	LEVEL 4	A	0.044363
0	24	LEVEL 4	B	0.039813
0	24	LEVEL 4	C	0.047142
0	24	LEVEL 5	A	0.022110
0	24	LEVEL 5	B	0.014020
0	24	LEVEL 5	C	0.019583
0	24	LEVEL 6	A	0.003971
0	24	LEVEL 6	B	0.003971
0	24	LEVEL 6	C	0.010932
24	48	CONTROL	A	0.056396
24	48	CONTROL	B	0.057126
24	48	CONTROL	C	0.061097
24	48	CONTROL	D	0.061496
24	48	CONTROL	E	0.057126
24	48	CONTROL	F	0.056588
24	48	LEVEL 1	A	0.058408
24	48	LEVEL 1	B	0.055073
24	48	LEVEL 1	C	0.050166
24	48	LEVEL 2	A	0.062152
24	48	LEVEL 2	B	0.059463
24	48	LEVEL 2	C	0.050166
24	48	LEVEL 3	A	0.046830
24	48	LEVEL 3	B	0.053040
24	48	LEVEL 3	C	0.065487
24	48	LEVEL 4	A	0.032983
24	48	LEVEL 4	B	0.044693
24	48	LEVEL 4	C	0.041535
24	48	LEVEL 5	A	0.027637
24	48	LEVEL 5	B	0.039353

ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
24	48	LEVEL 5	C	0.030163
24	48	LEVEL 6	A	0.015612
24	48	LEVEL 6	B	0.010048
24	48	LEVEL 6	C	0.000000
48	72	CONTROL	A	0.049111
48	72	CONTROL	B	0.044693
48	72	CONTROL	C	0.057762
48	72	CONTROL	D	0.057011
48	72	CONTROL	E	0.052654
48	72	CONTROL	F	0.055468
48	72	LEVEL 1	A	0.051738
48	72	LEVEL 1	B	0.054137
48	72	LEVEL 1	C	0.060288
48	72	LEVEL 2	A	0.050166
48	72	LEVEL 2	B	0.045776
48	72	LEVEL 2	C	0.059463
48	72	LEVEL 3	A	0.051194
48	72	LEVEL 3	B	0.048465
48	72	LEVEL 3	C	0.053550
48	72	LEVEL 4	A	0.067060
48	72	LEVEL 4	B	0.059899
48	72	LEVEL 4	C	0.048650
48	72	LEVEL 5	A	0.035304
48	72	LEVEL 5	B	0.041300
48	72	LEVEL 5	C	0.040868
48	72	LEVEL 6	A	-0.019583
48	72	LEVEL 6	B	-0.018875
48	72	LEVEL 6	C	-0.003335
72	96	CONTROL	A	0.052955
72	96	CONTROL	B	0.053736
72	96	CONTROL	C	0.050564
72	96	CONTROL	D	0.043705
72	96	CONTROL	E	0.050616
72	96	CONTROL	F	0.046554
72	96	LEVEL 1	A	0.046992
72	96	LEVEL 1	B	0.049976
72	96	LEVEL 1	C	0.046317
72	96	LEVEL 2	A	0.052495
72	96	LEVEL 2	B	0.052331
72	96	LEVEL 2	C	0.046601
72	96	LEVEL 3	A	0.058016
72	96	LEVEL 3	B	0.059359
72	96	LEVEL 3	C	0.048354
72	96	LEVEL 4	A	0.056774

ABC LABORATORIES, INC. 6
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
PRINTOUT OF RAW DATA

T1	TN	TREATMNT	REP	RATE
72	96	LEVEL 4	B	0.049111
72	96	LEVEL 4	C	0.051977
72	96	LEVEL 5	A	0.039771
72	96	LEVEL 5	B	0.030150
72	96	LEVEL 5	C	0.043505
72	96	LEVEL 6	A	0.000000
72	96	LEVEL 6	B	0.004856
72	96	LEVEL 6	C	0.011987

N = 96

ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=0 TN=24 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.045776	0.053372	0.049041	.002621399
LEVEL 1	3	0.048465	0.053372	0.050100	.002833419
LEVEL 2	3	0.041385	0.053372	0.049377	.006920555
LEVEL 3	3	0.041385	0.056707	0.046998	.008442714
LEVEL 4	3	0.039813	0.047142	0.043773	.003699887
LEVEL 5	3	0.014020	0.022110	0.018571	.004138884
LEVEL 6	3	0.003971	0.010932	0.006291	.004018697

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	5.3453	0.046290	0.051792
LEVEL 1	5.6555	0.043062	0.057139
LEVEL 2	14.0158	0.032185	0.066568
LEVEL 3	17.9640	0.026025	0.067971
LEVEL 4	8.4525	0.034582	0.052964
LEVEL 5	22.2869	0.008289	0.028852
LEVEL 6	63.8755	-0.003692	0.016274

----- T1=24 TN=48 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.056396	0.061496	0.058305	.002338832
LEVEL 1	3	0.050166	0.058408	0.054549	.004146294
LEVEL 2	3	0.050166	0.062152	0.057260	.006289671
LEVEL 3	3	0.046830	0.065487	0.055119	.009500671
LEVEL 4	3	0.032983	0.044693	0.039737	.006058645
LEVEL 5	3	0.027637	0.039353	0.032384	.006165389

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	4.0114	0.055851	0.060759
LEVEL 1	7.6010	0.044249	0.064849
LEVEL 2	10.9843	0.041636	0.072885
LEVEL 3	17.2365	0.031518	0.078720
LEVEL 4	15.2469	0.024686	0.054787
LEVEL 5	19.0382	0.017069	0.047700

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=24 TN=48 -----
 (continued)

T	R	N	M	M	S	L	U
E	O	I	A		T	W	P
A	N	X		D	E	E	
T	R	I	I	M	R	R	
M	E	M	M	E			
N	P	U	U	A	E	C	C
T	S	M	M	N	V	V	I

LEVEL 6 3 0 0.015612 .008553549 .007912736 92.5082 -0.011103 0.028210

----- T1=48 TN=72 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN
CONTROL	6	0.044693	0.057762	0.052783
LEVEL 1	3	0.051738	0.060288	0.055388
LEVEL 2	3	0.045776	0.059463	0.051801
LEVEL 3	3	0.048465	0.053550	0.051070
LEVEL 4	3	0.048650	0.067060	0.058537
LEVEL 5	3	0.035304	0.041300	0.039157
LEVEL 6	3	-0.019583	-0.003335	-0.013931

TREATMNT	STD_DEV	CV	LOWER_CI	UPPER_CI
CONTROL	.005076040	9.6168	0.047456	0.058110
LEVEL 1	.004410245	7.9625	0.044432	0.066343
LEVEL 2	.006988930	13.4918	0.034440	0.069163
LEVEL 3	.002544949	4.9833	0.044748	0.057392
LEVEL 4	.009280219	15.8537	0.035483	0.081590
LEVEL 5	.003343888	8.5396	0.030851	0.047464
LEVEL 6	.009183383	-65.9191	-0.036744	0.008882

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- T1=72 TN=96 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV
CONTROL	6	0.043705	0.053736	0.049688	.003855059
LEVEL 1	3	0.046317	0.049976	0.047761	.001947079
LEVEL 2	3	0.046601	0.052495	0.050475	.003356654
LEVEL 3	3	0.048354	0.059359	0.055243	.006003777
LEVEL 4	3	0.049111	0.056774	0.052621	.003872014
LEVEL 5	3	0.030150	0.043505	0.037809	.006890331
LEVEL 6	3	0.000000	0.011987	0.005614	.006029270

TREATMNT	CV	LOWER_CI	UPPER_CI
CONTROL	7.758	0.045643	0.053734
LEVEL 1	4.077	0.042925	0.052598
LEVEL 2	6.650	0.042137	0.058814
LEVEL 3	10.868	0.040328	0.070157
LEVEL 4	7.358	0.043002	0.062239
LEVEL 5	18.224	0.020692	0.054925
LEVEL 6	107.395	-0.009363	0.020592

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	2.89	0.0398
TRANSFORMED	6	17	9.82	0.0001

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 11
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Dependent Variable: RATE Values of RATE Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	774.666667	129.111111	5.99
Error	17	366.333333	21.549020	
Corrected Total	23	1141.000000		

Source	Pr > F
Model	0.0016
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.678937	37.13674	4.642092	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	774.666667	129.111111	5.99

Source	Pr > F
TREATMNT	0.0016

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=0 TN=24 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	21.54902
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	1.167	-8.365	10.698	
LEVEL 2 - CONTROL	0.500	-9.031	10.031	
LEVEL 3 - CONTROL	-2.500	-12.031	7.031	
LEVEL 4 - CONTROL	-6.167	-15.698	3.365	
LEVEL 5 - CONTROL	-11.667	-21.198	-2.135	***
LEVEL 6 - CONTROL	-14.667	-24.198	-5.135	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 0 AND ENDING HOUR= 24

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.049	0.003		
LEVEL 1	3	0.050	0.003	0.9990	
LEVEL 2	3	0.049	0.007	1.0000	
LEVEL 3	3	0.047	0.008	0.9527	
LEVEL 4	3	0.044	0.004	0.3222	
LEVEL 5	3	0.019	0.004	0.0130	*
LEVEL 6	3	0.006	0.004	0.0019	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 4.6421.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 24 AND ENDING HOUR= 48

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.62	0.2020
TRANSFORMED	6	17	1.65	0.1950

Assumptions of Normality and Homogeneity of Variance
 Are Met for the Raw Data Values.
 A Parametric Analysis is Performed on the Raw Data.

ABC LABORATORIES, INC. 16
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=24 TN=48 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=24 TN=48 -----

The GLM Procedure

Dependent Variable: RATE

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	0.00663278	0.00110546	31.53
Error	17	0.00059604	0.00003506	
Corrected Total	23	0.00722882		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.917547	13.00611	0.005921	0.045527

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	0.00663278	0.00110546	31.53

Source	Pr > F
TREATMNT	<.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2:3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=24 TN=48 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	0.000035
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
LEVEL 2 - CONTROL	-0.001045	-0.013203 0.011113	
LEVEL 3 - CONTROL	-0.003186	-0.015344 0.008972	
LEVEL 1 - CONTROL	-0.003756	-0.015914 0.008402	
LEVEL 4 - CONTROL	-0.018568	-0.030726 -0.006410	***
LEVEL 5 - CONTROL	-0.025921	-0.038078 -0.013763	***
LEVEL 6 - CONTROL	-0.049751	-0.061909 -0.037594	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 24 AND ENDING HOUR= 48

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.058	0.002		
LEVEL 1	3	0.055	0.004	0.9065	
LEVEL 2	3	0.057	0.006	0.9999	
LEVEL 3	3	0.055	0.010	0.9529	
LEVEL 4	3	0.040	0.006	0.0020	*
LEVEL 5	3	0.032	0.006	0.0001	*
LEVEL 6	3	0.009	0.008	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 0.0059.
 Assumptions of normality and homogeneity met.
 Analysis performed on raw data values.
 Minimally Significant Difference between the control mean and the treatment mean= 0.012

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 48 AND ENDING HOUR= 72

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.62	0.2027
TRANSFORMED	6	17	1.75	0.1700

Assumptions of Normality and Homogeneity of Variance
 Are Met for the Raw Data Values.
 A Parametric Analysis is Performed on the Raw Data.

ABC LABORATORIES, INC. 21
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=48 TN=72 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=48 TN=72 -----

The GLM Procedure

Dependent Variable: RATE

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	0.01194953	0.00199159	52.77
Error	17	0.00064165	0.00003774	
Corrected Total	23	0.01259118		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.949040	14.14005	0.006144	0.043448

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	0.01194953	0.00199159	52.77

Source	Pr > F
TREATMNT	<.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=48 TN=72 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	0.000038
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 4 - CONTROL	0.005753	-0.006861	0.018368	
LEVEL 1 - CONTROL	0.002604	-0.010010	0.015219	
LEVEL 2 - CONTROL	-0.000982	-0.013596	0.011633	
LEVEL 3 - CONTROL	-0.001714	-0.014328	0.010901	
LEVEL 5 - CONTROL	-0.013626	-0.026240	-0.001012	***
LEVEL 6 - CONTROL	-0.066715	-0.079329	-0.054100	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 48 AND ENDING HOUR= 72

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.053	0.005		
LEVEL 1	3	0.055	0.004	0.9845	
LEVEL 2	3	0.052	0.007	0.9999	
LEVEL 3	3	0.051	0.003	0.9983	
LEVEL 4	3	0.059	0.009	0.6618	
LEVEL 5	3	0.039	0.003	0.0311	*
LEVEL 6	3	-0.014	0.009	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 0.0061.
 Assumptions of normality and homogeneity met.
 Analysis performed on raw data values.
 Minimally Significant Difference between the control mean and the treatment mean= 0.013

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR BEGINNING HOUR= 72 AND ENDING HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.16	0.3705
TRANSFORMED	6	17	1.19	0.3560

Assumptions of Normality and Homogeneity of Variance
 Are Met for the Raw Data Values.
 A Parametric Analysis is Performed on the Raw Data.

ABC LABORATORIES, INC. 26
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=72 TN=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 27
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=72 TN=96 -----

The GLM Procedure

Dependent Variable: RATE

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	0.00549634	0.00091606	41.62
Error	17	0.00037416	0.00002201	
Corrected Total	23	0.00587050		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	RATE Mean
0.936265	10.75701	0.004691	0.043613

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	0.00549634	0.00091606	41.62

Source	Pr > F
TREATMNT	<.0001

ABC LABORATORIES, INC. 28
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- T1=72 TN=96 -----

The GLM Procedure

Dunnett's t Tests for RATE

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha 0.05
 Error Degrees of Freedom 17
 Error Mean Square 0.000022
 Critical Value of Dunnett's t 2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 3 - CONTROL	0.005554	-0.004078	0.015187	
LEVEL 4 - CONTROL	0.002932	-0.006700	0.012565	
LEVEL 2 - CONTROL	0.000787	-0.008846	0.010420	
LEVEL 1 - CONTROL	-0.001927	-0.011559	0.007706	
LEVEL 5 - CONTROL	-0.011879	-0.021512	-0.002247	***
LEVEL 6 - CONTROL	-0.044074	-0.053707	-0.034442	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN
 ANALYSIS OF GROWTH RATE BETWEEN ADJACENT TIME PERIODS
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 PARAMETRIC ANALYSIS
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR BEGINNING HOUR= 72 AND ENDING HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	0.050	0.004		
LEVEL 1	3	0.048	0.002	0.9868	
LEVEL 2	3	0.050	0.003	0.9999	
LEVEL 3	3	0.055	0.006	0.4344	
LEVEL 4	3	0.053	0.004	0.9118	
LEVEL 5	3	0.038	0.007	0.0123	*
LEVEL 6	3	0.006	0.006	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 0.0047.
 Assumptions of normality and homogeneity met.
 Analysis performed on raw data values.
 Minimally Significant Difference between the control mean and the treatment mean= 0.010

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405GROWTH.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK

ON: 13OCT09 *MBE*
(3 Oct 09)

THE ANALYSIS WAS REVIEWED

BY: *MBE* ON: 22 Oct 09

ABC LABORATORIES, INC. 1
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	2.1
24	CONTROL	B	2.3
24	CONTROL	C	2.0
24	CONTROL	D	2.2
24	CONTROL	E	2.3
24	CONTROL	F	2.6
24	LEVEL 1	A	2.2
24	LEVEL 1	B	2.2
24	LEVEL 1	C	2.6
24	LEVEL 2	A	1.7
24	LEVEL 2	B	2.6
24	LEVEL 2	C	2.6
24	LEVEL 3	A	2.9
24	LEVEL 3	B	1.8
24	LEVEL 3	C	1.7
24	LEVEL 4	A	1.9
24	LEVEL 4	B	1.6
24	LEVEL 4	C	2.1
24	LEVEL 5	A	0.7
24	LEVEL 5	B	0.4
24	LEVEL 5	C	0.6
24	LEVEL 6	A	0.1
24	LEVEL 6	B	0.1
24	LEVEL 6	C	0.3
48	CONTROL	A	11.0
48	CONTROL	B	12.0
48	CONTROL	C	12.0
48	CONTROL	D	13.0
48	CONTROL	E	12.0
48	CONTROL	F	13.0
48	LEVEL 1	A	12.0
48	LEVEL 1	B	11.0
48	LEVEL 1	C	11.0
48	LEVEL 2	A	11.0
48	LEVEL 2	B	14.0
48	LEVEL 2	C	11.0
48	LEVEL 3	A	11.0
48	LEVEL 3	B	9.0

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
48	LEVEL 3	C	12.00
48	LEVEL 4	A	5.40
48	LEVEL 4	B	6.60
48	LEVEL 4	C	7.40
48	LEVEL 5	A	2.30
48	LEVEL 5	B	2.60
48	LEVEL 5	C	2.30
48	LEVEL 6	A	0.60
48	LEVEL 6	B	0.40
48	LEVEL 6	C	0.30
72	CONTROL	A	38.00
72	CONTROL	B	37.00
72	CONTROL	C	51.00
72	CONTROL	D	54.00
72	CONTROL	E	45.00
72	CONTROL	F	52.00
72	LEVEL 1	A	44.00
72	LEVEL 1	B	43.00
72	LEVEL 1	C	50.00
72	LEVEL 2	A	39.00
72	LEVEL 2	B	44.00
72	LEVEL 2	C	49.00
72	LEVEL 3	A	40.00
72	LEVEL 3	B	31.00
72	LEVEL 3	C	46.00
72	LEVEL 4	A	31.00
72	LEVEL 4	B	31.00
72	LEVEL 4	C	26.00
72	LEVEL 5	A	6.70
72	LEVEL 5	B	8.70
72	LEVEL 5	C	7.80
72	LEVEL 6	A	0.00
72	LEVEL 6	B	-0.11
72	LEVEL 6	C	0.20
96	CONTROL	A	138.00
96	CONTROL	B	137.00
96	CONTROL	C	174.00
96	CONTROL	D	156.00
96	CONTROL	E	154.00
96	CONTROL	F	161.00
96	LEVEL 1	A	138.00
96	LEVEL 1	B	145.00
96	LEVEL 1	C	154.00
96	LEVEL 2	A	140.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
96	LEVEL 2	B	157.0
96	LEVEL 2	C	152.0
96	LEVEL 3	A	164.0
96	LEVEL 3	B	132.0
96	LEVEL 3	C	149.0
96	LEVEL 4	A	124.0
96	LEVEL 4	B	103.0
96	LEVEL 4	C	93.0
96	LEVEL 5	A	19.0
96	LEVEL 5	B	19.0
96	LEVEL 5	C	24.0
96	LEVEL 6	A	0.0
96	LEVEL 6	B	0.0
96	LEVEL 6	C	0.6

N = 102

ABC LABORATORIES, INC. 4
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=0 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV	CV	LOWER_CI	UPPER_CI
CONTROL	6	1	1	1	0	0	1	1

----- HOUR=24 -----

T	N	M	M		S		L	U
R	O	I	A		T		O	P
A	R	N	X		D		W	P
T	I	I		M			E	E
M	E	M	M	E	D		R	R
N	P	U	U	A	E	C	C	C
T	S	M	M	N	V	V	I	I
CONTROL	6	2.0	2.6	2.25000	0.20736	9.2162	2.03238	2.46762
LEVEL 1	3	2.2	2.6	2.33333	0.23094	9.8974	1.75965	2.90702
LEVEL 2	3	1.7	2.6	2.30000	0.51962	22.5920	1.00920	3.59080
LEVEL 3	3	1.7	2.9	2.13333	0.66583	31.2109	0.47931	3.78735
LEVEL 4	3	1.6	2.1	1.86667	0.25166	13.4818	1.24151	2.49183
LEVEL 5	3	0.4	0.7	0.56667	0.15275	26.9563	0.18721	0.94612
LEVEL 6	3	0.1	0.3	0.16667	0.11547	69.2820	-0.12018	0.45351

----- HOUR=48 -----

T	N	M	M		S		L	U
R	O	I	A		T		O	P
A	R	N	X		D		W	P
T	I	I		M			E	E
M	E	M	M	E	D		R	R
N	P	U	U	A	E	C	C	C
T	S	M	M	N	V	V	I	I
CONTROL	6	11.0	13.0	12.1667	0.75277	6.1872	11.3767	12.9567
LEVEL 1	3	11.0	12.0	11.3333	0.57735	5.0943	9.8991	12.7676
LEVEL 2	3	11.0	14.0	12.0000	1.73205	14.4338	7.6973	16.3027
LEVEL 3	3	9.0	12.0	10.6667	1.52753	14.3205	6.8721	14.4612
LEVEL 4	3	5.4	7.4	6.4667	1.00664	15.5667	3.9660	8.9673

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=48 -----
 (continued)

	T R E A T M E N T	N	M I N	M A X		S T D D E V		C V		L O W E R C I	U P P E R C I
LEVEL 5	3	2.3	2.6	2.40000	0.17321	7.2169	1.96973	2.83027			
LEVEL 6	3	0.3	0.6	0.43333	0.15275	35.2506	0.05388	0.81279			

----- HOUR=72 -----

	T R E A T M E N T	N	M I N	M A X		S T D D E V		C V		L O W E R C I	U P P E R C I
CONTROL	6	37.00	54.0	46.1667	7.35980	15.942	38.4430	53.8903			
LEVEL 1	3	43.00	50.0	45.6667	3.78594	8.290	36.2619	55.0715			
LEVEL 2	3	39.00	49.0	44.0000	5.00000	11.364	31.5793	56.4207			
LEVEL 3	3	31.00	46.0	39.0000	7.54983	19.359	20.2452	57.7548			
LEVEL 4	3	26.00	31.0	29.3333	2.88675	9.841	22.1622	36.5044			
LEVEL 5	3	6.70	8.7	7.7333	1.00167	12.953	5.2451	10.2216			
LEVEL 6	3	-0.11	0.2	0.0300	0.15716	523.874	-0.3604	0.4204			

ABC LABORATORIES, INC. 6
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=96 -----									
T								L	U
R	N	M	M		S			O	P
E	O	I	A		T			W	P
A		N	X		D			E	E
T	R	I	I	M				R	R
M	E	M	M	E	D				
N	P	U	U	A	E	C		C	C
T	S	M	M	N	V	V		I	I
CONTROL	6	137	174.0	153.333	14.1091	9.202		138.527	168.140
LEVEL 1	3	138	154.0	145.667	8.0208	5.506		125.742	165.591
LEVEL 2	3	140	157.0	149.667	8.7369	5.838		127.963	171.370
LEVEL 3	3	132	164.0	148.333	16.0104	10.794		108.561	188.105
LEVEL 4	3	93	124.0	106.667	15.8219	14.833		67.363	145.971
LEVEL 5	3	19	24.0	20.667	2.8868	13.968		13.496	27.838
LEVEL 6	3	0	0.6	0.200	0.3464	173.205		-0.661	1.061

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 24

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	4.53	0.0064
TRANSFORMED	6	17	2.86	0.0408

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 8
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=24 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=24 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	774.666667	129.111111	5.99
Error	17	366.333333	21.549020	
Corrected Total	23	1141.000000		

Source	Pr > F
Model	0.0016
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.678937	37.13674	4.642092	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	774.666667	129.111111	5.99

Source	Pr > F
TREATMNT	0.0016

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=24 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	21.54902
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	1.167	-8.365	10.698	
LEVEL 2 - CONTROL	0.500	-9.031	10.031	
LEVEL 3 - CONTROL	-2.500	-12.031	7.031	
LEVEL 4 - CONTROL	-6.167	-15.698	3.365	
LEVEL 5 - CONTROL	-11.667	-21.198	-2.135	***
LEVEL 6 - CONTROL	-14.667	-24.198	-5.135	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 24

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	2.25	0.21		
LEVEL 1	3	2.33	0.23	0.9990	
LEVEL 2	3	2.30	0.52	1.0000	
LEVEL 3	3	2.13	0.67	0.9527	
LEVEL 4	3	1.87	0.25	0.3222	
LEVEL 5	3	0.57	0.15	0.0130	*
LEVEL 6	3	0.17	0.12	0.0019	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 4.6421.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 48

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.42	0.0211
TRANSFORMED	6	17	2.07	0.1119

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 13
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=48 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 14
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=48 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	926.791667	154.465278	13.49
Error	17	194.708333	11.453431	
Corrected Total	23	1121.500000		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.826386	27.07433	3.384292	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	926.7916667	154.4652778	13.49

Source	Pr > F
TREATMNT	<.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=48 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	11.45343
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-2.250	-9.199	4.699	
LEVEL 1 - CONTROL	-3.917	-10.865	3.032	
LEVEL 3 - CONTROL	-5.083	-12.032	1.865	
LEVEL 4 - CONTROL	-11.250	-18.199	-4.301	***
LEVEL 5 - CONTROL	-14.250	-21.199	-7.301	***
LEVEL 6 - CONTROL	-17.250	-24.199	-10.301	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 48

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	12.17	0.75		
LEVEL 1	3	11.33	0.58	0.4573	
LEVEL 2	3	12.00	1.73	0.8879	
LEVEL 3	3	10.67	1.53	0.2162	
LEVEL 4	3	6.47	1.01	0.0012	*
LEVEL 5	3	2.40	0.17	0.0001	*
LEVEL 6	3	0.43	0.15	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.3843.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

ABC LABORATORIES, INC. 17
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 72

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	<0.01	<0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.51	0.0191
TRANSFORMED	6	16	1.69	0.1871

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 18
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=72 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 19
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=72 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	885.500000	147.583333	9.58
Error	17	262.000000	15.411765	
Corrected Total	23	1147.500000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.771678	31.40626	3.925782	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	885.500000	147.583333	9.58

Source	Pr > F
TREATMNT	0.0001

ABC LABORATORIES, INC. 20
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=72 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.41176
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	-0.833	-8.894	7.227	
LEVEL 2 - CONTROL	-1.833	-9.894	6.227	
LEVEL 3 - CONTROL	-4.333	-12.394	3.727	
LEVEL 4 - CONTROL	-10.000	-18.061	-1.939	***
LEVEL 5 - CONTROL	-13.333	-21.394	-5.273	***
LEVEL 6 - CONTROL	-16.333	-24.394	-8.273	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 72

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	46.17	7.36		
LEVEL 1	3	45.67	3.79	0.9996	
LEVEL 2	3	44.00	5.00	0.9753	
LEVEL 3	3	39.00	7.55	0.5048	
LEVEL 4	3	29.33	2.89	0.0118	*
LEVEL 5	3	7.73	1.00	0.0009	*
LEVEL 6	3	0.03	0.16	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9258.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

ABC LABORATORIES, INC. 22
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.72	0.1778
TRANSFORMED	6	17	0.68	0.6644

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 23
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 24
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	880.000000	146.666667	9.30
Error	17	268.000000	15.764706	
Corrected Total	23	1148.000000		

Source	Pr > F
Model	0.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.766551	31.76383	3.970479	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	880.000000	146.666667	9.30

Source	Pr > F
TREATMNT	0.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.76471
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-0.667	-8.819	7.486	
LEVEL 3 - CONTROL	-1.667	-9.819	6.486	
LEVEL 1 - CONTROL	-2.667	-10.819	5.486	
LEVEL 4 - CONTROL	-10.000	-18.152	-1.848	***
LEVEL 5 - CONTROL	-13.000	-21.152	-4.848	***
LEVEL 6 - CONTROL	-16.000	-24.152	-7.848	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	153.33	14.11		
LEVEL 1	3	145.67	8.02	0.8835	
LEVEL 2	3	149.67	8.74	0.9999	
LEVEL 3	3	148.33	16.01	0.9852	
LEVEL 4	3	106.67	15.82	0.0128	*
LEVEL 5	3	20.67	2.89	0.0013	*
LEVEL 6	3	0.20	0.35	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9705.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

ABC LABORATORIES, INC. 27
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=24 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.0	A	2.1	2.25	6.7
CONTROL	0.0	B	2.3	2.25	-2.1
CONTROL	0.0	C	2.0	2.25	11.1
CONTROL	0.0	D	2.2	2.25	2.2
CONTROL	0.0	E	2.3	2.25	-2.1
CONTROL	0.0	F	2.6	2.25	-15.5
LEVEL 1	2.5	A	2.2	2.25	2.2
LEVEL 1	2.5	B	2.2	2.25	2.2
LEVEL 1	2.5	C	2.6	2.25	-15.5
LEVEL 2	5.0	A	1.7	2.25	24.4
LEVEL 2	5.0	B	2.6	2.25	-15.5
LEVEL 2	5.0	C	2.6	2.25	-15.5
LEVEL 3	10.0	A	2.9	2.25	-28.8
LEVEL 3	10.0	B	1.8	2.25	20.0
LEVEL 3	10.0	C	1.7	2.25	24.4
LEVEL 4	20.0	A	1.9	2.25	15.6
LEVEL 4	20.0	B	1.6	2.25	28.9
LEVEL 4	20.0	C	2.1	2.25	6.7
LEVEL 5	40.0	A	0.7	2.25	68.9
LEVEL 5	40.0	B	0.4	2.25	82.2
LEVEL 5	40.0	C	0.6	2.25	73.3
LEVEL 6	80.0	A	0.1	2.25	95.6
LEVEL 6	80.0	B	0.1	2.25	95.6
LEVEL 6	80.0	C	0.3	2.25	86.7

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=48 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.0	A	11.0	12.1667	9.6
CONTROL	0.0	B	12.0	12.1667	1.4
CONTROL	0.0	C	12.0	12.1667	1.4
CONTROL	0.0	D	13.0	12.1667	-6.7
CONTROL	0.0	E	12.0	12.1667	1.4
CONTROL	0.0	F	13.0	12.1667	-6.7
LEVEL 1	2.5	A	12.0	12.1667	1.4
LEVEL 1	2.5	B	11.0	12.1667	9.6
LEVEL 1	2.5	C	11.0	12.1667	9.6
LEVEL 2	5.0	A	11.0	12.1667	9.6
LEVEL 2	5.0	B	14.0	12.1667	-15.0
LEVEL 2	5.0	C	11.0	12.1667	9.6
LEVEL 3	10.0	A	11.0	12.1667	9.6
LEVEL 3	10.0	B	9.0	12.1667	26.0
LEVEL 3	10.0	C	12.0	12.1667	1.4
LEVEL 4	20.0	A	5.4	12.1667	55.6
LEVEL 4	20.0	B	6.6	12.1667	45.8
LEVEL 4	20.0	C	7.4	12.1667	39.2
LEVEL 5	40.0	A	2.3	12.1667	81.1
LEVEL 5	40.0	B	2.6	12.1667	78.6
LEVEL 5	40.0	C	2.3	12.1667	81.1
LEVEL 6	80.0	A	0.6	12.1667	95.1
LEVEL 6	80.0	B	0.4	12.1667	96.7
LEVEL 6	80.0	C	0.3	12.1667	97.5

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=72 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.0	A	38.00	46.1667	17.7
CONTROL	0.0	B	37.00	46.1667	19.9
CONTROL	0.0	C	51.00	46.1667	-10.4
CONTROL	0.0	D	54.00	46.1667	-16.9
CONTROL	0.0	E	45.00	46.1667	2.5
CONTROL	0.0	F	52.00	46.1667	-12.5
LEVEL 1	2.5	A	44.00	46.1667	4.7
LEVEL 1	2.5	B	43.00	46.1667	6.9
LEVEL 1	2.5	C	50.00	46.1667	-8.2
LEVEL 2	5.0	A	39.00	46.1667	15.5
LEVEL 2	5.0	B	44.00	46.1667	4.7
LEVEL 2	5.0	C	49.00	46.1667	-6.0
LEVEL 3	10.0	A	40.00	46.1667	13.4
LEVEL 3	10.0	B	31.00	46.1667	32.9
LEVEL 3	10.0	C	46.00	46.1667	0.4
LEVEL 4	20.0	A	31.00	46.1667	32.9
LEVEL 4	20.0	B	31.00	46.1667	32.9
LEVEL 4	20.0	C	26.00	46.1667	43.7
LEVEL 5	40.0	A	6.70	46.1667	85.5
LEVEL 5	40.0	B	8.70	46.1667	81.2
LEVEL 5	40.0	C	7.80	46.1667	83.1
LEVEL 6	80.0	A	0.00	46.1667	100.0
LEVEL 6	80.0	B	-0.11	46.1667	100.2
LEVEL 6	80.0	C	0.20	46.1667	99.6

N = 24

ABC LABORATORIES, INC. 30
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=96 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.0	A	138.0	153.333	10.0
CONTROL	0.0	B	137.0	153.333	10.7
CONTROL	0.0	C	174.0	153.333	-13.4
CONTROL	0.0	D	156.0	153.333	-1.6
CONTROL	0.0	E	154.0	153.333	-0.3
CONTROL	0.0	F	161.0	153.333	-4.9
LEVEL 1	2.5	A	138.0	153.333	10.0
LEVEL 1	2.5	B	145.0	153.333	5.4
LEVEL 1	2.5	C	154.0	153.333	-0.3
LEVEL 2	5.0	A	140.0	153.333	8.7
LEVEL 2	5.0	B	157.0	153.333	-2.3
LEVEL 2	5.0	C	152.0	153.333	0.9
LEVEL 3	10.0	A	164.0	153.333	-6.9
LEVEL 3	10.0	B	132.0	153.333	13.9
LEVEL 3	10.0	C	149.0	153.333	2.8
LEVEL 4	20.0	A	124.0	153.333	19.1
LEVEL 4	20.0	B	103.0	153.333	32.8
LEVEL 4	20.0	C	93.0	153.333	39.3
LEVEL 5	40.0	A	19.0	153.333	87.6
LEVEL 5	40.0	B	19.0	153.333	87.6
LEVEL 5	40.0	C	24.0	153.333	84.3
LEVEL 6	80.0	A	0.0	153.333	100.0
LEVEL 6	80.0	B	0.0	153.333	100.0
LEVEL 6	80.0	C	0.6	153.333	99.6

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=24 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-15.5	11.1	0.0	9.2	-9.6	9.7
LEVEL 1	2.5	3	-15.5	2.2	-3.6	10.3	-29.1	21.8
LEVEL 2	5.0	3	-15.5	24.4	-2.1	23.1	-59.5	55.1
LEVEL 3	10.0	3	-28.8	24.4	5.2	29.6	-68.2	78.7
LEVEL 4	20.0	3	6.7	28.9	17.0	11.2	-10.6	44.8
LEVEL 5	40.0	3	68.9	82.2	74.8	6.8	58.0	91.7
LEVEL 6	80.0	3	86.7	95.6	92.6	5.1	79.8	105.3

----- HOUR=48 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-6.7	9.6	0.0	6.2	-6.4	6.5
LEVEL 1	2.5	3	1.4	9.6	6.8	4.7	-4.8	18.6
LEVEL 2	5.0	3	-15.0	9.6	1.4	14.2	-33.9	36.7
LEVEL 3	10.0	3	1.4	26.0	12.3	12.6	-18.8	43.5
LEVEL 4	20.0	3	39.2	55.6	46.8	8.3	26.3	67.4
LEVEL 5	40.0	3	78.6	81.1	80.3	1.4	76.7	83.8
LEVEL 6	80.0	3	95.1	97.5	96.4	1.3	93.3	99.6

----- HOUR=72 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-16.9	19.9	0.0	15.9	-16.6	16.7
LEVEL 1	2.5	3	-8.2	6.9	1.1	8.2	-19.2	21.5
LEVEL 2	5.0	3	-6.0	15.5	4.7	10.8	-22.1	31.6
LEVEL 3	10.0	3	0.4	32.9	15.5	16.4	-25.0	56.1
LEVEL 4	20.0	3	32.9	43.7	36.5	6.3	20.9	52.0
LEVEL 5	40.0	3	81.2	85.5	83.2	2.2	77.9	88.6
LEVEL 6	80.0	3	99.6	100.2	99.9	0.3	99.1	100.8

ABC LABORATORIES, INC. 32
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=96 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.0	6	-13.4	10.7	0.0	9.2	-9.6	9.7
LEVEL 1	2.5	3	-0.3	10.0	5.0	5.2	-7.9	18.0
LEVEL 2	5.0	3	-2.3	8.7	2.4	5.7	-11.7	16.5
LEVEL 3	10.0	3	-6.9	13.9	3.3	10.4	-22.6	29.2
LEVEL 4	20.0	3	19.1	39.3	30.4	10.3	4.8	56.1
LEVEL 5	40.0	3	84.3	87.6	86.5	1.9	81.8	91.2
LEVEL 6	80.0	3	99.6	100.0	99.9	0.2	99.3	100.4

ABC LABORATORIES, INC. 33
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 24

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.36 AND EC50= 29.744370251

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 117.46 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 15.01
 R-SQUARED= 88.00

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 12.393431251 95% LOWER LIMIT= 8.536461177 95% UPPER LIMIT= 16.250401325
PERCENT= 10	EC= 15.476715605 95% LOWER LIMIT= 11.575498923 95% UPPER LIMIT= 19.377932287
PERCENT= 20	EC= 19.69670671 95% LOWER LIMIT= 15.958228981 95% UPPER LIMIT= 23.435184439
PERCENT= 50	EC= 29.744370251 95% LOWER LIMIT= 26.797647927 95% UPPER LIMIT= 32.691092574
PERCENT= 90	EC= 57.165071982 95% LOWER LIMIT= 48.665659922 95% UPPER LIMIT= 65.664484041

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 48

ESTIMATES OF COEFFICIENTS:
 SLOPE= 2.34 AND EC50= 21.456983819

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 371.52 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 8.12
 R-SQUARED= 95.90

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 6.0988434844 95% LOWER LIMIT= 4.6607204927 95% UPPER LIMIT= 7.5369664761
PERCENT= 10	EC= 8.3924387475 95% LOWER LIMIT= 6.8064200818 95% UPPER LIMIT= 9.9784574132
PERCENT= 20	EC= 11.867272651 95% LOWER LIMIT= 10.203680724 95% UPPER LIMIT= 13.530864578
PERCENT= 50	EC= 21.456983819 95% LOWER LIMIT= 19.865247292 95% UPPER LIMIT= 23.048720346
PERCENT= 90	EC= 54.859161736 95% LOWER LIMIT= 47.604488729 95% UPPER LIMIT= 62.113834742

ABC LABORATORIES, INC. 35
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 72

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.14 AND EC50= 23.770262605

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 317.68 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 9.08
 R-SQUARED= 95.20

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 9.3194413593 95% LOWER LIMIT= 7.8353310883 95% UPPER LIMIT= 10.80355163
PERCENT= 10	EC= 11.819103137 95% LOWER LIMIT= 10.303602355 95% UPPER LIMIT= 13.334603918
PERCENT= 20	EC= 15.296028651 95% LOWER LIMIT= 13.823417812 95% UPPER LIMIT= 16.76863949
PERCENT= 50	EC= 23.770262605 95% LOWER LIMIT= 22.457863725 95% UPPER LIMIT= 25.082661486
PERCENT= 90	EC= 47.806113357 95% LOWER LIMIT= 43.319711046 95% UPPER LIMIT= 52.292515668

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 96

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.91 AND EC50= 24.753156197

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 721.64 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 6.37
 R-SQUARED= 97.80

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 11.645855887 95% LOWER LIMIT= 10.288712228 95% UPPER LIMIT= 13.002999545
PERCENT= 10	EC= 14.101690665 95% LOWER LIMIT= 12.764503648 95% UPPER LIMIT= 15.438877683
PERCENT= 20	EC= 17.356292766 95% LOWER LIMIT= 16.092002843 95% UPPER LIMIT= 18.620582689
PERCENT= 50	EC= 24.753156197 95% LOWER LIMIT= 23.611880249 95% UPPER LIMIT= 25.894432146
PERCENT= 90	EC= 43.450020021 95% LOWER LIMIT= 40.331751096 95% UPPER LIMIT= 46.568288945

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 13OCT09
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK

ON: 13OCT09 *MRP*
13 Oct 09

THE ANALYSIS WAS REVIEWED

BY: *MRP*

ON: 22 Oct 09

ABC LABORATORIES, INC. 1
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	2.1
24	CONTROL	B	2.3
24	CONTROL	C	2.0
24	CONTROL	D	2.2
24	CONTROL	E	2.3
24	CONTROL	F	2.6
24	LEVEL 1	A	2.2
24	LEVEL 1	B	2.2
24	LEVEL 1	C	2.6
24	LEVEL 2	A	1.7
24	LEVEL 2	B	2.6
24	LEVEL 2	C	2.6
24	LEVEL 3	A	2.9
24	LEVEL 3	B	1.8
24	LEVEL 3	C	1.7
24	LEVEL 4	A	1.9
24	LEVEL 4	B	1.6
24	LEVEL 4	C	2.1
24	LEVEL 5	A	0.7
24	LEVEL 5	B	0.4
24	LEVEL 5	C	0.6
24	LEVEL 6	A	0.1
24	LEVEL 6	B	0.1
24	LEVEL 6	C	0.3
48	CONTROL	A	11.0
48	CONTROL	B	12.0
48	CONTROL	C	12.0
48	CONTROL	D	13.0
48	CONTROL	E	12.0
48	CONTROL	F	13.0
48	LEVEL 1	A	12.0
48	LEVEL 1	B	11.0
48	LEVEL 1	C	11.0
48	LEVEL 2	A	11.0
48	LEVEL 2	B	14.0
48	LEVEL 2	C	11.0
48	LEVEL 3	A	11.0
48	LEVEL 3	B	9.0

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
48	LEVEL 3	C	12.00
48	LEVEL 4	A	5.40
48	LEVEL 4	B	6.60
48	LEVEL 4	C	7.40
48	LEVEL 5	A	2.30
48	LEVEL 5	B	2.60
48	LEVEL 5	C	2.30
48	LEVEL 6	A	0.60
48	LEVEL 6	B	0.40
48	LEVEL 6	C	0.30
72	CONTROL	A	38.00
72	CONTROL	B	37.00
72	CONTROL	C	51.00
72	CONTROL	D	54.00
72	CONTROL	E	45.00
72	CONTROL	F	52.00
72	LEVEL 1	A	44.00
72	LEVEL 1	B	43.00
72	LEVEL 1	C	50.00
72	LEVEL 2	A	39.00
72	LEVEL 2	B	44.00
72	LEVEL 2	C	49.00
72	LEVEL 3	A	40.00
72	LEVEL 3	B	31.00
72	LEVEL 3	C	46.00
72	LEVEL 4	A	31.00
72	LEVEL 4	B	31.00
72	LEVEL 4	C	26.00
72	LEVEL 5	A	6.70
72	LEVEL 5	B	8.70
72	LEVEL 5	C	7.80
72	LEVEL 6	A	0.00
72	LEVEL 6	B	-0.11
72	LEVEL 6	C	0.20
96	CONTROL	A	138.00
96	CONTROL	B	137.00
96	CONTROL	C	174.00
96	CONTROL	D	156.00
96	CONTROL	E	154.00
96	CONTROL	F	161.00
96	LEVEL 1	A	138.00
96	LEVEL 1	B	145.00
96	LEVEL 1	C	154.00
96	LEVEL 2	A	140.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
96	LEVEL 2	B	157.0
96	LEVEL 2	C	152.0
96	LEVEL 3	A	164.0
96	LEVEL 3	B	132.0
96	LEVEL 3	C	149.0
96	LEVEL 4	A	124.0
96	LEVEL 4	B	103.0
96	LEVEL 4	C	93.0
96	LEVEL 5	A	19.0
96	LEVEL 5	B	19.0
96	LEVEL 5	C	24.0
96	LEVEL 6	A	0.0
96	LEVEL 6	B	0.0
96	LEVEL 6	C	0.6

N = 102

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=0 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV	CV	LOWER_CI	UPPER_CI
CONTROL	6	1	1	1	0	0	1	1

----- HOUR=24 -----

T	N	M	M		S		L	U
R	O	I	A		T		O	P
E							W	P
A	N	X			D		E	E
T	R	I	I	M			R	R
M	E	M	M	E	D			
N	P	U	U	A	E	C	C	C
T	S	M	M	N	V	V	I	I
CONTROL	6	2.0	2.6	2.25000	0.20736	9.2162	2.03238	2.46762
LEVEL 1	3	2.2	2.6	2.33333	0.23094	9.8974	1.75965	2.90702
LEVEL 2	3	1.7	2.6	2.30000	0.51962	22.5920	1.00920	3.59080
LEVEL 3	3	1.7	2.9	2.13333	0.66583	31.2109	0.47931	3.78735
LEVEL 4	3	1.6	2.1	1.86667	0.25166	13.4818	1.24151	2.49183
LEVEL 5	3	0.4	0.7	0.56667	0.15275	26.9563	0.18721	0.94612
LEVEL 6	3	0.1	0.3	0.16667	0.11547	69.2820	-0.12018	0.45351

----- HOUR=48 -----

T	N	M	M		S		L	U
R	O	I	A		T		O	P
E							W	P
A	N	X			D		E	E
T	R	I	I	M			R	R
M	E	M	M	E	D			
N	P	U	U	A	E	C	C	C
T	S	M	M	N	V	V	I	I
CONTROL	6	11.0	13.0	12.1667	0.75277	6.1872	11.3767	12.9567
LEVEL 1	3	11.0	12.0	11.3333	0.57735	5.0943	9.8991	12.7676
LEVEL 2	3	11.0	14.0	12.0000	1.73205	14.4338	7.6973	16.3027
LEVEL 3	3	9.0	12.0	10.6667	1.52753	14.3205	6.8721	14.4612
LEVEL 4	3	5.4	7.4	6.4667	1.00664	15.5667	3.9660	8.9673

ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=48 -----
 (continued)

T							L	U
R	N	M	M		S		O	P
E	O	I	A		T		W	P
A		N	X		D		E	E
T	R	I	I	M			R	R
M	E	M	M	E	D			
N	P	U	U	A	E	C	C	C
T	S	M	M	N	V	V	I	I

LEVEL 5	3	2.3	2.6	2.40000	0.17321	7.2169	1.96973	2.83027
LEVEL 6	3	0.3	0.6	0.43333	0.15275	35.2506	0.05388	0.81279

----- HOUR=72 -----

T							L	U
R	N	M	M		S		O	P
E	O	I	A		T		W	P
A		N	X		D		E	E
T	R	I	I	M			R	R
M	E	M	M	E	D			
N	P	U	U	A	E	C	C	C
T	S	M	M	N	V	V	I	I

CONTROL	6	37.00	54.0	46.1667	7.35980	15.942	38.4430	53.8903
LEVEL 1	3	43.00	50.0	45.6667	3.78594	8.290	36.2619	55.0715
LEVEL 2	3	39.00	49.0	44.0000	5.00000	11.364	31.5793	56.4207
LEVEL 3	3	31.00	46.0	39.0000	7.54983	19.359	20.2452	57.7548
LEVEL 4	3	26.00	31.0	29.3333	2.88675	9.841	22.1622	36.5044
LEVEL 5	3	6.70	8.7	7.7333	1.00167	12.953	5.2451	10.2216
LEVEL 6	3	-0.11	0.2	0.0300	0.15716	523.874	-0.3604	0.4204

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=96 -----

T	N	M	M	S	L	U		
R	O	I	A	T	O	P		
A	N	X		D	W	P		
T	R	I	I	M	E	E		
M	E	M	M	E	R	R		
N	P	U	U	A	C	C		
T	S	M	M	N	V	I		
CONTROL	6	137	174.0	153.333	14.1091	9.202	138.527	168.140
LEVEL 1	3	138	154.0	145.667	8.0208	5.506	125.742	165.591
LEVEL 2	3	140	157.0	149.667	8.7369	5.838	127.963	171.370
LEVEL 3	3	132	164.0	148.333	16.0104	10.794	108.561	188.105
LEVEL 4	3	93	124.0	106.667	15.8219	14.833	67.363	145.971
LEVEL 5	3	19	24.0	20.667	2.8868	13.968	13.496	27.838
LEVEL 6	3	0	0.6	0.200	0.3464	173.205	-0.661	1.061

ABC LABORATORIES, INC.
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 24

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	4.53	0.0064
TRANSFORMED	6	17	2.86	0.0408

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 8
 SAS PROGRAM AL ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=24 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=24 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	774.666667	129.111111	5.99
Error	17	366.333333	21.549020	
Corrected Total	23	1141.000000		

Source	Pr > F
Model	0.0016
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.678937	37.13674	4.642092	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	774.666667	129.111111	5.99

Source	Pr > F
TREATMNT	0.0016

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=24 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	21.54902
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	1.167	-8.365	10.698	
LEVEL 2 - CONTROL	0.500	-9.031	10.031	
LEVEL 3 - CONTROL	-2.500	-12.031	7.031	
LEVEL 4 - CONTROL	-6.167	-15.698	3.365	
LEVEL 5 - CONTROL	-11.667	-21.198	-2.135	***
LEVEL 6 - CONTROL	-14.667	-24.198	-5.135	***

ABC LABORATORIES, INC. 11
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 24

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	2.25	0.21		
LEVEL 1	3	2.33	0.23	0.9990	
LEVEL 2	3	2.30	0.52	1.0000	
LEVEL 3	3	2.13	0.67	0.9527	
LEVEL 4	3	1.87	0.25	0.3222	
LEVEL 5	3	0.57	0.15	0.0130	*
LEVEL 6	3	0.17	0.12	0.0019	*

Note: * indicates significant differences from
 control at the 0.05 level using a
 two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees
 of freedom, and error root mean square= 4.6421.
 Analysis performed on ranks of values. Least
 significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 48

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	<0.01	<0.01
LEVEL 2	3	<0.01	<0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.42	0.0211
TRANSFORMED	6	17	2.07	0.1119

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 13
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=48 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=48 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	926.791667	154.465278	13.49
Error	17	194.708333	11.453431	
Corrected Total	23	1121.500000		

Source	Pr > F
Model	<.0001
Error	
Corrected Total	

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.826386	27.07433	3.384292	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	926.7916667	154.4652778	13.49

Source	Pr > F
TREATMNT	<.0001

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=48 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for
 comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	11.45343
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-2.250	-9.199	4.699	
LEVEL 1 - CONTROL	-3.917	-10.865	3.032	
LEVEL 3 - CONTROL	-5.083	-12.032	1.865	
LEVEL 4 - CONTROL	-11.250	-18.199	-4.301	***
LEVEL 5 - CONTROL	-14.250	-21.199	-7.301	***
LEVEL 6 - CONTROL	-17.250	-24.199	-10.301	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 48

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	12.17	0.75		
LEVEL 1	3	11.33	0.58	0.4573	
LEVEL 2	3	12.00	1.73	0.8879	
LEVEL 3	3	10.67	1.53	0.2162	
LEVEL 4	3	6.47	1.01	0.0012	*
LEVEL 5	3	2.40	0.17	0.0001	*
LEVEL 6	3	0.43	0.15	0.0000	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.3843.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 72

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	<0.01	<0.01
LEVEL 5	3	>0.01	>0.01
LEVEL 6	3	>0.01	>0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	3.51	0.0191
TRANSFORMED	6	16	1.69	0.1871

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 18
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=72 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6
		Number of Observations Read 24
		Number of Observations Used 24

ABC LABORATORIES, INC. 19
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=72 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	885.500000	147.583333	9.58
Error	17	262.000000	15.411765	
Corrected Total	23	1147.500000		

Source Pr > F

Model 0.0001

Error

Corrected Total

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.771678	31.40626	3.925782	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	885.500000	147.583333	9.58

Source Pr > F

TREATMNT 0.0001

ABC LABORATORIES, INC. 20
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=72 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.41176
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 1 - CONTROL	-0.833	-8.894	7.227	
LEVEL 2 - CONTROL	-1.833	-9.894	6.227	
LEVEL 3 - CONTROL	-4.333	-12.394	3.727	
LEVEL 4 - CONTROL	-10.000	-18.061	-1.939	***
LEVEL 5 - CONTROL	-13.333	-21.394	-5.273	***
LEVEL 6 - CONTROL	-16.333	-24.394	-8.273	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 72

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	46.17	7.36		
LEVEL 1	3	45.67	3.79	0.9996	
LEVEL 2	3	44.00	5.00	0.9753	
LEVEL 3	3	39.00	7.55	0.5048	
LEVEL 4	3	29.33	2.89	0.0118	*
LEVEL 5	3	7.73	1.00	0.0009	*
LEVEL 6	3	0.03	0.16	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9258.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA p VALUE	TRANSFORMED p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.72	0.1778
TRANSFORMED	6	17	0.68	0.6644

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 23
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6

Number of Observations Read	24
Number of Observations Used	24

ABC LABORATORIES, INC. 24
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	880.000000	146.666667	9.30
Error	17	268.000000	15.764706	
Corrected Total	23	1148.000000		

Source Pr > F

Model 0.0001

Error

Corrected Total

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.766551	31.76383	3.970479	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	880.000000	146.666667	9.30

Source Pr > F

TREATMNT 0.0001

ABC LABORATORIES, INC. 25
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.76471
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-0.667	-8.819	7.486	
LEVEL 3 - CONTROL	-1.667	-9.819	6.486	
LEVEL 1 - CONTROL	-2.667	-10.819	5.486	
LEVEL 4 - CONTROL	-10.000	-18.152	-1.848	***
LEVEL 5 - CONTROL	-13.000	-21.152	-4.848	***
LEVEL 6 - CONTROL	-16.000	-24.152	-7.848	***

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	153.33	14.11		
LEVEL 1	3	145.67	8.02	0.8835	
LEVEL 2	3	149.67	8.74	0.9999	
LEVEL 3	3	148.33	16.01	0.9852	
LEVEL 4	3	106.67	15.82	0.0128	*
LEVEL 5	3	20.67	2.89	0.0013	*
LEVEL 6	3	0.20	0.35	0.0001	*

Note: * indicates significant differences from control at the 0.05 level using a two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees of freedom, and error root mean square= 3.9705.
 Analysis performed on ranks of values. Least significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=24 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	2.1	2.25	6.7
CONTROL	0.00	B	2.3	2.25	-2.1
CONTROL	0.00	C	2.0	2.25	11.1
CONTROL	0.00	D	2.2	2.25	2.2
CONTROL	0.00	E	2.3	2.25	-2.1
CONTROL	0.00	F	2.6	2.25	-15.5
LEVEL 1	1.69	A	2.2	2.25	2.2
LEVEL 1	1.69	B	2.2	2.25	2.2
LEVEL 1	1.69	C	2.6	2.25	-15.5
LEVEL 2	3.48	A	1.7	2.25	24.4
LEVEL 2	3.48	B	2.6	2.25	-15.5
LEVEL 2	3.48	C	2.6	2.25	-15.5
LEVEL 3	7.38	A	2.9	2.25	-28.8
LEVEL 3	7.38	B	1.8	2.25	20.0
LEVEL 3	7.38	C	1.7	2.25	24.4
LEVEL 4	15.00	A	1.9	2.25	15.6
LEVEL 4	15.00	B	1.6	2.25	28.9
LEVEL 4	15.00	C	2.1	2.25	6.7
LEVEL 5	28.90	A	0.7	2.25	68.9
LEVEL 5	28.90	B	0.4	2.25	82.2
LEVEL 5	28.90	C	0.6	2.25	73.3
LEVEL 6	44.90	A	0.1	2.25	95.6
LEVEL 6	44.90	B	0.1	2.25	95.6
LEVEL 6	44.90	C	0.3	2.25	86.7

N = 24

ABC LABORATORIES, INC. 28
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=48 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	11.0	12.1667	9.6
CONTROL	0.00	B	12.0	12.1667	1.4
CONTROL	0.00	C	12.0	12.1667	1.4
CONTROL	0.00	D	13.0	12.1667	-6.7
CONTROL	0.00	E	12.0	12.1667	1.4
CONTROL	0.00	F	13.0	12.1667	-6.7
LEVEL 1	1.69	A	12.0	12.1667	1.4
LEVEL 1	1.69	B	11.0	12.1667	9.6
LEVEL 1	1.69	C	11.0	12.1667	9.6
LEVEL 2	3.48	A	11.0	12.1667	9.6
LEVEL 2	3.48	B	14.0	12.1667	-15.0
LEVEL 2	3.48	C	11.0	12.1667	9.6
LEVEL 3	7.38	A	11.0	12.1667	9.6
LEVEL 3	7.38	B	9.0	12.1667	26.0
LEVEL 3	7.38	C	12.0	12.1667	1.4
LEVEL 4	15.00	A	5.4	12.1667	55.6
LEVEL 4	15.00	B	6.6	12.1667	45.8
LEVEL 4	15.00	C	7.4	12.1667	39.2
LEVEL 5	28.90	A	2.3	12.1667	81.1
LEVEL 5	28.90	B	2.6	12.1667	78.6
LEVEL 5	28.90	C	2.3	12.1667	81.1
LEVEL 6	44.90	A	0.6	12.1667	95.1
LEVEL 6	44.90	B	0.4	12.1667	96.7
LEVEL 6	44.90	C	0.3	12.1667	97.5

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=72 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	38.00	46.1667	17.7
CONTROL	0.00	B	37.00	46.1667	19.9
CONTROL	0.00	C	51.00	46.1667	-10.4
CONTROL	0.00	D	54.00	46.1667	-16.9
CONTROL	0.00	E	45.00	46.1667	2.5
CONTROL	0.00	F	52.00	46.1667	-12.5
LEVEL 1	1.69	A	44.00	46.1667	4.7
LEVEL 1	1.69	B	43.00	46.1667	6.9
LEVEL 1	1.69	C	50.00	46.1667	-8.2
LEVEL 2	3.48	A	39.00	46.1667	15.5
LEVEL 2	3.48	B	44.00	46.1667	4.7
LEVEL 2	3.48	C	49.00	46.1667	-6.0
LEVEL 3	7.38	A	40.00	46.1667	13.4
LEVEL 3	7.38	B	31.00	46.1667	32.9
LEVEL 3	7.38	C	46.00	46.1667	0.4
LEVEL 4	15.00	A	31.00	46.1667	32.9
LEVEL 4	15.00	B	31.00	46.1667	32.9
LEVEL 4	15.00	C	26.00	46.1667	43.7
LEVEL 5	28.90	A	6.70	46.1667	85.5
LEVEL 5	28.90	B	8.70	46.1667	81.2
LEVEL 5	28.90	C	7.80	46.1667	83.1
LEVEL 6	44.90	A	0.00	46.1667	100.0
LEVEL 6	44.90	B	-0.11	46.1667	100.2
LEVEL 6	44.90	C	0.20	46.1667	99.6

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=96 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	138.0	153.333	10.0
CONTROL	0.00	B	137.0	153.333	10.7
CONTROL	0.00	C	174.0	153.333	-13.4
CONTROL	0.00	D	156.0	153.333	-1.6
CONTROL	0.00	E	154.0	153.333	-0.3
CONTROL	0.00	F	161.0	153.333	-4.9
LEVEL 1	1.69	A	138.0	153.333	10.0
LEVEL 1	1.69	B	145.0	153.333	5.4
LEVEL 1	1.69	C	154.0	153.333	-0.3
LEVEL 2	3.48	A	140.0	153.333	8.7
LEVEL 2	3.48	B	157.0	153.333	-2.3
LEVEL 2	3.48	C	152.0	153.333	0.9
LEVEL 3	7.38	A	164.0	153.333	-6.9
LEVEL 3	7.38	B	132.0	153.333	13.9
LEVEL 3	7.38	C	149.0	153.333	2.8
LEVEL 4	15.00	A	124.0	153.333	19.1
LEVEL 4	15.00	B	103.0	153.333	32.8
LEVEL 4	15.00	C	93.0	153.333	39.3
LEVEL 5	28.90	A	19.0	153.333	87.6
LEVEL 5	28.90	B	19.0	153.333	87.6
LEVEL 5	28.90	C	24.0	153.333	84.3
LEVEL 6	44.90	A	0.0	153.333	100.0
LEVEL 6	44.90	B	0.0	153.333	100.0
LEVEL 6	44.90	C	0.6	153.333	99.6

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=24 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-15.5	11.1	0.0	9.2	-9.6	9.7
LEVEL 1	1.69	3	-15.5	2.2	-3.6	10.3	-29.1	21.8
LEVEL 2	3.48	3	-15.5	24.4	-2.1	23.1	-59.5	55.1
LEVEL 3	7.38	3	-28.8	24.4	5.2	29.6	-68.2	78.7
LEVEL 4	15.00	3	6.7	28.9	17.0	11.2	-10.6	44.8
LEVEL 5	28.90	3	68.9	82.2	74.8	6.8	58.0	91.7
LEVEL 6	44.90	3	86.7	95.6	92.6	5.1	79.8	105.3

----- HOUR=48 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-6.7	9.6	0.0	6.2	-6.4	6.5
LEVEL 1	1.69	3	1.4	9.6	6.8	4.7	-4.8	18.6
LEVEL 2	3.48	3	-15.0	9.6	1.4	14.2	-33.9	36.7
LEVEL 3	7.38	3	1.4	26.0	12.3	12.6	-18.8	43.5
LEVEL 4	15.00	3	39.2	55.6	46.8	8.3	26.3	67.4
LEVEL 5	28.90	3	78.6	81.1	80.3	1.4	76.7	83.8
LEVEL 6	44.90	3	95.1	97.5	96.4	1.3	93.3	99.6

----- HOUR=72 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-16.9	19.9	0.0	15.9	-16.6	16.7
LEVEL 1	1.69	3	-8.2	6.9	1.1	8.2	-19.2	21.5
LEVEL 2	3.48	3	-6.0	15.5	4.7	10.8	-22.1	31.6
LEVEL 3	7.38	3	0.4	32.9	15.5	16.4	-25.0	56.1
LEVEL 4	15.00	3	32.9	43.7	36.5	6.3	20.9	52.0
LEVEL 5	28.90	3	81.2	85.5	83.2	2.2	77.9	88.6
LEVEL 6	44.90	3	99.6	100.2	99.9	0.3	99.1	100.8

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=96 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-13.4	10.7	0.0	9.2	-9.6	9.7
LEVEL 1	1.69	3	-0.3	10.0	5.0	5.2	-7.9	18.0
LEVEL 2	3.48	3	-2.3	8.7	2.4	5.7	-11.7	16.5
LEVEL 3	7.38	3	-6.9	13.9	3.3	10.4	-22.6	29.2
LEVEL 4	15.00	3	19.1	39.3	30.4	10.3	4.8	56.1
LEVEL 5	28.90	3	84.3	87.6	86.5	1.9	81.8	91.2
LEVEL 6	44.90	3	99.6	100.0	99.9	0.2	99.3	100.4

ABC LABORATORIES, INC. 33
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 24

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.83 AND EC50= 21.998102742

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 120 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 14.87
 R-SQUARED= 88.20

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 10.198540657
	95% LOWER LIMIT= 7.7733068195
	95% UPPER LIMIT= 12.623774495
PERCENT= 10	EC= 12.395353063
	95% LOWER LIMIT= 9.9819773397
	95% UPPER LIMIT= 14.808728786
PERCENT= 20	EC= 15.31806879
	95% LOWER LIMIT= 13.033502917
	95% UPPER LIMIT= 17.602634662
PERCENT= 50	EC= 21.998102742
	95% LOWER LIMIT= 20.228311541
	95% UPPER LIMIT= 23.767893942
PERCENT= 90	EC= 39.040156563
	95% LOWER LIMIT= 35.149311561
	95% UPPER LIMIT= 42.931001565

ABC LABORATORIES, INC. 34
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 48

ESTIMATES OF COEFFICIENTS:
 SLOPE= 2.60 AND EC50= 15.948308345

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 362.53 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 8.21
 R-SQUARED= 95.80

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 5.13484701 95% LOWER LIMIT= 3.9832563881 95% UPPER LIMIT= 6.2864376318
PERCENT= 10	EC= 6.8458995401 95% LOWER LIMIT= 5.6074020072 95% UPPER LIMIT= 8.084397073
PERCENT= 20	EC= 9.3537154876 95% LOWER LIMIT= 8.0849980433 95% UPPER LIMIT= 10.622432932
PERCENT= 50	EC= 15.948308345 95% LOWER LIMIT= 14.785811299 95% UPPER LIMIT= 17.110805391
PERCENT= 90	EC= 37.15341389 95% LOWER LIMIT= 32.744566579 95% UPPER LIMIT= 41.562261202

ABC LABORATORIES, INC. 35
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 72

ESTIMATES OF COEFFICIENTS:
 SLOPE= 3.51 AND EC50= 17.669683473

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 283.93 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 9.57
 R-SQUARED= 94.70

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 7.6375037895 95% LOWER LIMIT= 6.4748046093 95% UPPER LIMIT= 8.8002029697
PERCENT= 10	EC= 9.4491905513 95% LOWER LIMIT= 8.2830836926 95% UPPER LIMIT= 10.61529741
PERCENT= 20	EC= 11.904756272 95% LOWER LIMIT= 10.787828943 95% UPPER LIMIT= 13.021683602
PERCENT= 50	EC= 17.669683473 95% LOWER LIMIT= 16.684147342 95% UPPER LIMIT= 18.655219603
PERCENT= 90	EC= 33.041741758 95% LOWER LIMIT= 30.127641951 95% UPPER LIMIT= 35.955841566

ABC LABORATORIES, INC. 36
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 96

ESTIMATES OF COEFFICIENTS:
 SLOPE= 4.23 AND EC50= 18.330855899

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 701.7 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 6.46
 R-SQUARED= 97.80

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 9.1378697894 95% LOWER LIMIT= 8.1152046054 95% UPPER LIMIT= 10.160534973
PERCENT= 10	EC= 10.903593359 95% LOWER LIMIT= 9.9056998319 95% UPPER LIMIT= 11.901486887
PERCENT= 20	EC= 13.207989888 95% LOWER LIMIT= 12.271173177 95% UPPER LIMIT= 14.144806599
PERCENT= 50	EC= 18.330855899 95% LOWER LIMIT= 17.490117217 95% UPPER LIMIT= 19.171594581
PERCENT= 90	EC= 30.817388993 95% LOWER LIMIT= 28.709329626 95% UPPER LIMIT= 32.925448361

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK ON: 14JUL10
MRE 14JUL10

THE ANALYSIS WAS REVIEWED

BY: AAL ON: 20SEP10

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
0	CONTROL	A	1.0
0	CONTROL	B	1.0
0	CONTROL	C	1.0
0	CONTROL	D	1.0
0	CONTROL	E	1.0
0	CONTROL	F	1.0
24	CONTROL	A	2.1
24	CONTROL	B	2.3
24	CONTROL	C	2.0
24	CONTROL	D	2.2
24	CONTROL	E	2.3
24	CONTROL	F	2.6
24	LEVEL 1	A	2.2
24	LEVEL 1	B	2.2
24	LEVEL 1	C	2.6
24	LEVEL 2	A	1.7
24	LEVEL 2	B	2.6
24	LEVEL 2	C	2.6
24	LEVEL 3	A	2.9
24	LEVEL 3	B	1.8
24	LEVEL 3	C	1.7
24	LEVEL 4	A	1.9
24	LEVEL 4	B	1.6
24	LEVEL 4	C	2.1
24	LEVEL 5	A	0.7
24	LEVEL 5	B	0.4
24	LEVEL 5	C	0.6
24	LEVEL 6	A	0.1
24	LEVEL 6	B	0.1
24	LEVEL 6	C	0.3
48	CONTROL	A	11.0
48	CONTROL	B	12.0
48	CONTROL	C	12.0
48	CONTROL	D	13.0
48	CONTROL	E	12.0
48	CONTROL	F	13.0
48	LEVEL 1	A	12.0
48	LEVEL 1	B	11.0
48	LEVEL 1	C	11.0
48	LEVEL 2	A	11.0
48	LEVEL 2	B	14.0
48	LEVEL 2	C	11.0
48	LEVEL 3	A	11.0
48	LEVEL 3	B	9.0

ABC LABORATORIES, INC. 2
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
48	LEVEL 3	C	12.00
48	LEVEL 4	A	5.40
48	LEVEL 4	B	6.60
48	LEVEL 4	C	7.40
48	LEVEL 5	A	2.30
48	LEVEL 5	B	2.60
48	LEVEL 5	C	2.30
48	LEVEL 6	A	0.60
48	LEVEL 6	B	0.40
48	LEVEL 6	C	0.30
72	CONTROL	A	38.00
72	CONTROL	B	37.00
72	CONTROL	C	51.00
72	CONTROL	D	54.00
72	CONTROL	E	45.00
72	CONTROL	F	52.00
72	LEVEL 1	A	44.00
72	LEVEL 1	B	43.00
72	LEVEL 1	C	50.00
72	LEVEL 2	A	39.00
72	LEVEL 2	B	44.00
72	LEVEL 2	C	49.00
72	LEVEL 3	A	40.00
72	LEVEL 3	B	31.00
72	LEVEL 3	C	46.00
72	LEVEL 4	A	31.00
72	LEVEL 4	B	31.00
72	LEVEL 4	C	26.00
72	LEVEL 5	A	6.70
72	LEVEL 5	B	8.70
72	LEVEL 5	C	7.80
72	LEVEL 6	A	0.00
72	LEVEL 6	B	-0.11
72	LEVEL 6	C	0.20
96	CONTROL	A	138.00
96	CONTROL	B	137.00
96	CONTROL	C	174.00
96	CONTROL	D	156.00
96	CONTROL	E	154.00
96	CONTROL	F	161.00
96	LEVEL 1	A	138.00
96	LEVEL 1	B	145.00
96	LEVEL 1	C	154.00
96	LEVEL 2	A	140.00

ABC LABORATORIES, INC. 3
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
PRINTOUT OF RAW DATA

HOUR	TREATMNT	REP	CELL_104
96	LEVEL 2	B	157.0
96	LEVEL 2	C	152.0
96	LEVEL 3	A	164.0
96	LEVEL 3	B	132.0
96	LEVEL 3	C	149.0
96	LEVEL 4	A	124.0
96	LEVEL 4	B	103.0
96	LEVEL 4	C	93.0
96	LEVEL 5	A	19.0
96	LEVEL 5	B	19.0
96	LEVEL 5	C	24.0
96	LEVEL 6	A	0.0
96	LEVEL 6	B	0.0
96	LEVEL 6	C	0.6

N = 102

ABC LABORATORIES, INC. 4
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=0 -----

TREATMNT	NO_REPS	MINIMUM	MAXIMUM	MEAN	STD_DEV	CV	LOWER_CI	UPPER_CI
CONTROL	6	1	1	1	0	0	1	1

----- HOUR=24 -----

T	R	N	M	M	S	L	U	
E	O	I	A	T	W	P		
A	—	N	X	D	E	E		
T	R	I	I	M	R	R		
M	E	M	M	E	D			
N	P	U	U	A	E	C	C	
T	S	M	M	N	V	V	I	
CONTROL	6	2.0	2.6	2.25000	0.20736	9.2162	2.03238	2.46762
LEVEL 1	3	2.2	2.6	2.33333	0.23094	9.8974	1.75965	2.90702
LEVEL 2	3	1.7	2.6	2.30000	0.51962	22.5920	1.00920	3.59080
LEVEL 3	3	1.7	2.9	2.13333	0.66583	31.2109	0.47931	3.78735
LEVEL 4	3	1.6	2.1	1.86667	0.25166	13.4818	1.24151	2.49183
LEVEL 5	3	0.4	0.7	0.56667	0.15275	26.9563	0.18721	0.94612
LEVEL 6	3	0.1	0.3	0.16667	0.11547	69.2820	-0.12018	0.45351

----- HOUR=48 -----

T	R	N	M	M	S	L	U	
E	O	I	A	T	W	P		
A	—	N	X	D	E	E		
T	R	I	I	M	R	R		
M	E	M	M	E	D			
N	P	U	U	A	E	C	C	
T	S	M	M	N	V	V	I	
CONTROL	6	11.0	13.0	12.1667	0.75277	6.1872	11.3767	12.9567
LEVEL 1	3	11.0	12.0	11.3333	0.57735	5.0943	9.8991	12.7676
LEVEL 2	3	11.0	14.0	12.0000	1.73205	14.4338	7.6973	16.3027
LEVEL 3	3	9.0	12.0	10.6667	1.52753	14.3205	6.8721	14.4612
LEVEL 4	3	5.4	7.4	6.4667	1.00664	15.5667	3.9660	8.9673

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=48 -----
 (continued)

	T R E A T M E N T	N	M I N	M A X	M E A N	S T D E V	C O V	L O W E R C I	U P P E R C I
LEVEL 5	3	2.3	2.6	2.40000	0.17321	7.2169	1.96973	2.83027	
LEVEL 6	3	0.3	0.6	0.43333	0.15275	35.2506	0.05388	0.81279	

----- HOUR=72 -----

	T R E A T M E N T	N	M I N	M A X	M E A N	S T D E V	C O V	L O W E R C I	U P P E R C I
CONTROL	6	37.00	54.0	46.1667	7.35980	15.942	38.4430	53.8903	
LEVEL 1	3	43.00	50.0	45.6667	3.78594	8.290	36.2619	55.0715	
LEVEL 2	3	39.00	49.0	44.0000	5.00000	11.364	31.5793	56.4207	
LEVEL 3	3	31.00	46.0	39.0000	7.54983	19.359	20.2452	57.7548	
LEVEL 4	3	26.00	31.0	29.3333	2.88675	9.841	22.1622	36.5044	
LEVEL 5	3	6.70	8.7	7.7333	1.00167	12.953	5.2451	10.2216	
LEVEL 6	3	-0.11	0.2	0.0300	0.15716	523.874	-0.3604	0.4204	

ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, CV,
 AND LOWER AND UPPER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=96 -----

T	N	M	M	S	L	U		
R	O	I	A	T	O	P		
E		N	X	D	W	P		
A	R	I	I	M	E	E		
T	E	M	M	E	R	R		
M	P	U	U	A	C	C		
N	S	M	M	N	V	V		
T					I	I		
CONTROL	6	137	174.0	153.333	14.1091	9.202	138.527	168.140
LEVEL 1	3	138	154.0	145.667	8.0208	5.506	125.742	165.591
LEVEL 2	3	140	157.0	149.667	8.7369	5.838	127.963	171.370
LEVEL 3	3	132	164.0	148.333	16.0104	10.794	108.561	188.105
LEVEL 4	3	93	124.0	106.667	15.8219	14.833	67.363	145.971
LEVEL 5	3	19	24.0	20.667	2.8868	13.968	13.496	27.838
LEVEL 6	3	0	0.6	0.200	0.3464	173.205	-0.661	1.061

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 RESULTS OF TESTS FOR NORMALITY & HOMOGENEITY OF VARIANCE

FOR HOUR= 96

Results of Shapiro-Wilk Test for Normality Conducted
 on Rep Residuals for each Treatment.
 P value <0.01 indicates Nonnormality.
 P value >0.01 indicates Normality.

GROUP	NO. OF REPS	RAW DATA	TRANSFORMED
		p VALUE	p VALUE
CONTROL	6	>0.01	>0.01
LEVEL 1	3	>0.01	>0.01
LEVEL 2	3	>0.01	>0.01
LEVEL 3	3	>0.01	>0.01
LEVEL 4	3	>0.01	>0.01
LEVEL 5	3	<0.01	<0.01
LEVEL 6	3	<0.01	<0.01

Results of Levene's Test for Homogeneity of Variance
 Conducted on Rep Residuals for each Treatment.
 P value less than 0.01 indicates Unequal Treatment Variances.
 P value greater than 0.01 indicates Equal Treatment Variances.

VARIABLE	DEGREES OF FREEDOM		F	p VALUE
	NUMERATOR	DENOMINATOR		
RAW DATA	6	17	1.72	0.1778
TRANSFORMED	6	17	0.68	0.6644

Assumptions of Normality and Homogeneity of Variance
 Are Not Met for the Raw or Transformed Values.
 A Nonparametric Analysis is Performed on the Ranks of the Data.

ABC LABORATORIES, INC. 8
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Class Level Information

Class	Levels	Values
TREATMNT	7	CONTROL LEVEL 1 LEVEL 2 LEVEL 3 LEVEL 4 LEVEL 5 LEVEL 6
		Number of Observations Read 24
		Number of Observations Used 24

ABC LABORATORIES, INC. 9
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Dependent Variable: CELL_104 Values of CELL_104 Were Replaced by Ranks

Source	DF	Sum of Squares	Mean Square	F Value
Model	6	880.000000	146.666667	9.30
Error	17	268.000000	15.764706	
Corrected Total	23	1148.000000		

Source Pr > F

Model 0.0001

Error

Corrected Total

R-Square	Coeff Var	Root MSE	CELL_104 Mean
0.766551	31.76383	3.970479	12.50000

Source	DF	Type III SS	Mean Square	F Value
TREATMNT	6	880.000000	146.666667	9.30

Source Pr > F

TREATMNT 0.0001

ABC LABORATORIES, INC. 10
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DUNNETT'S 2-TAILED TEST OF DIFFERENCES FROM THE CONTROL

----- HOUR=96 -----

The GLM Procedure

Dunnett's t Tests for CELL_104

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	17
Error Mean Square	15.76471
Critical Value of Dunnett's t	2.90374

Comparisons significant at the 0.05 level are indicated by ***.

TREATMNT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
LEVEL 2 - CONTROL	-0.667	-8.819	7.486	
LEVEL 3 - CONTROL	-1.667	-9.819	6.486	
LEVEL 1 - CONTROL	-2.667	-10.819	5.486	
LEVEL 4 - CONTROL	-10.000	-18.152	-1.848	***
LEVEL 5 - CONTROL	-13.000	-21.152	-4.848	***
LEVEL 6 - CONTROL	-16.000	-24.152	-7.848	***

ABC LABORATORIES, INC. 11
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 COMPARING THE TREATMENT GROUPS TO THE CONTROL
 NONPARAMETRIC ANALYSIS ON RANKS OF DATA
 ANOVA AND MEANS COMPARISON AT THE 0.05 LEVEL
 DESCRIPTIVE STATISTICS AND RESULTS OF DUNNETT'S TEST

FOR HOUR= 96

GROUP	n	MEAN	STD.DEV.	p	SIG.
CONTROL	6	153.33	14.11		
LEVEL 1	3	145.67	8.02	0.8835	
LEVEL 2	3	149.67	8.74	0.9999	
LEVEL 3	3	148.33	16.01	0.9852	
LEVEL 4	3	106.67	15.82	0.0128	*
LEVEL 5	3	20.67	2.89	0.0013	*
LEVEL 6	3	0.20	0.35	0.0001	*

Note: * indicates significant differences from
 control at the 0.05 level using a
 two-tailed Dunnett's test.
 Results based on 3 reps, 17 error degrees
 of freedom, and error root mean square= 3.9705.
 Analysis performed on ranks of values. Least
 significant difference cannot be computed.

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=24 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	2.1	2.25	6.7
CONTROL	0.00	B	2.3	2.25	-2.1
CONTROL	0.00	C	2.0	2.25	11.1
CONTROL	0.00	D	2.2	2.25	2.2
CONTROL	0.00	E	2.3	2.25	-2.1
CONTROL	0.00	F	2.6	2.25	-15.5
LEVEL 1	1.64	A	2.2	2.25	2.2
LEVEL 1	1.64	B	2.2	2.25	2.2
LEVEL 1	1.64	C	2.6	2.25	-15.5
LEVEL 2	3.51	A	1.7	2.25	24.4
LEVEL 2	3.51	B	2.6	2.25	-15.5
LEVEL 2	3.51	C	2.6	2.25	-15.5
LEVEL 3	7.41	A	2.9	2.25	-28.8
LEVEL 3	7.41	B	1.8	2.25	20.0
LEVEL 3	7.41	C	1.7	2.25	24.4
LEVEL 4	14.80	A	1.9	2.25	15.6
LEVEL 4	14.80	B	1.6	2.25	28.9
LEVEL 4	14.80	C	2.1	2.25	6.7
LEVEL 5	28.40	A	0.7	2.25	68.9
LEVEL 5	28.40	B	0.4	2.25	82.2
LEVEL 5	28.40	C	0.6	2.25	73.3
LEVEL 6	44.80	A	0.1	2.25	95.6
LEVEL 6	44.80	B	0.1	2.25	95.6
LEVEL 6	44.80	C	0.3	2.25	86.7

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=48 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	11.0	12.1667	9.6
CONTROL	0.00	B	12.0	12.1667	1.4
CONTROL	0.00	C	12.0	12.1667	1.4
CONTROL	0.00	D	13.0	12.1667	-6.7
CONTROL	0.00	E	12.0	12.1667	1.4
CONTROL	0.00	F	13.0	12.1667	-6.7
LEVEL 1	1.64	A	12.0	12.1667	1.4
LEVEL 1	1.64	B	11.0	12.1667	9.6
LEVEL 1	1.64	C	11.0	12.1667	9.6
LEVEL 2	3.51	A	11.0	12.1667	9.6
LEVEL 2	3.51	B	14.0	12.1667	-15.0
LEVEL 2	3.51	C	11.0	12.1667	9.6
LEVEL 3	7.41	A	11.0	12.1667	9.6
LEVEL 3	7.41	B	9.0	12.1667	26.0
LEVEL 3	7.41	C	12.0	12.1667	1.4
LEVEL 4	14.80	A	5.4	12.1667	55.6
LEVEL 4	14.80	B	6.6	12.1667	45.8
LEVEL 4	14.80	C	7.4	12.1667	39.2
LEVEL 5	28.40	A	2.3	12.1667	81.1
LEVEL 5	28.40	B	2.6	12.1667	78.6
LEVEL 5	28.40	C	2.3	12.1667	81.1
LEVEL 6	44.80	A	0.6	12.1667	95.1
LEVEL 6	44.80	B	0.4	12.1667	96.7
LEVEL 6	44.80	C	0.3	12.1667	97.5

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=72 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	38.00	46.1667	17.7
CONTROL	0.00	B	37.00	46.1667	19.9
CONTROL	0.00	C	51.00	46.1667	-10.4
CONTROL	0.00	D	54.00	46.1667	-16.9
CONTROL	0.00	E	45.00	46.1667	2.5
CONTROL	0.00	F	52.00	46.1667	-12.5
LEVEL 1	1.64	A	44.00	46.1667	4.7
LEVEL 1	1.64	B	43.00	46.1667	6.9
LEVEL 1	1.64	C	50.00	46.1667	-8.2
LEVEL 2	3.51	A	39.00	46.1667	15.5
LEVEL 2	3.51	B	44.00	46.1667	4.7
LEVEL 2	3.51	C	49.00	46.1667	-6.0
LEVEL 3	7.41	A	40.00	46.1667	13.4
LEVEL 3	7.41	B	31.00	46.1667	32.9
LEVEL 3	7.41	C	46.00	46.1667	0.4
LEVEL 4	14.80	A	31.00	46.1667	32.9
LEVEL 4	14.80	B	31.00	46.1667	32.9
LEVEL 4	14.80	C	26.00	46.1667	43.7
LEVEL 5	28.40	A	6.70	46.1667	85.5
LEVEL 5	28.40	B	8.70	46.1667	81.2
LEVEL 5	28.40	C	7.80	46.1667	83.1
LEVEL 6	44.80	A	0.00	46.1667	100.0
LEVEL 6	44.80	B	-0.11	46.1667	100.2
LEVEL 6	44.80	C	0.20	46.1667	99.6

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 PRINTOUT OF PERCENT INHIBITION VALUES

----- HOUR=96 -----

TREATMNT	CONC	REP	CELL_104	CON_MEAN	PER_INH
CONTROL	0.00	A	138.0	153.333	10.0
CONTROL	0.00	B	137.0	153.333	10.7
CONTROL	0.00	C	174.0	153.333	-13.4
CONTROL	0.00	D	156.0	153.333	-1.6
CONTROL	0.00	E	154.0	153.333	-0.3
CONTROL	0.00	F	161.0	153.333	-4.9
LEVEL 1	1.64	A	138.0	153.333	10.0
LEVEL 1	1.64	B	145.0	153.333	5.4
LEVEL 1	1.64	C	154.0	153.333	-0.3
LEVEL 2	3.51	A	140.0	153.333	8.7
LEVEL 2	3.51	B	157.0	153.333	-2.3
LEVEL 2	3.51	C	152.0	153.333	0.9
LEVEL 3	7.41	A	164.0	153.333	-6.9
LEVEL 3	7.41	B	132.0	153.333	13.9
LEVEL 3	7.41	C	149.0	153.333	2.8
LEVEL 4	14.80	A	124.0	153.333	19.1
LEVEL 4	14.80	B	103.0	153.333	32.8
LEVEL 4	14.80	C	93.0	153.333	39.3
LEVEL 5	28.40	A	19.0	153.333	87.6
LEVEL 5	28.40	B	19.0	153.333	87.6
LEVEL 5	28.40	C	24.0	153.333	84.3
LEVEL 6	44.80	A	0.0	153.333	100.0
LEVEL 6	44.80	B	0.0	153.333	100.0
LEVEL 6	44.80	C	0.6	153.333	99.6

N = 24

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=24 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-15.5	11.1	0.0	9.2	-9.6	9.7
LEVEL 1	1.64	3	-15.5	2.2	-3.6	10.3	-29.1	21.8
LEVEL 2	3.51	3	-15.5	24.4	-2.1	23.1	-59.5	55.1
LEVEL 3	7.41	3	-28.8	24.4	5.2	29.6	-68.2	78.7
LEVEL 4	14.80	3	6.7	28.9	17.0	11.2	-10.6	44.8
LEVEL 5	28.40	3	68.9	82.2	74.8	6.8	58.0	91.7
LEVEL 6	44.80	3	86.7	95.6	92.6	5.1	79.8	105.3

----- HOUR=48 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-6.7	9.6	0.0	6.2	-6.4	6.5
LEVEL 1	1.64	3	1.4	9.6	6.8	4.7	-4.8	18.6
LEVEL 2	3.51	3	-15.0	9.6	1.4	14.2	-33.9	36.7
LEVEL 3	7.41	3	1.4	26.0	12.3	12.6	-18.8	43.5
LEVEL 4	14.80	3	39.2	55.6	46.8	8.3	26.3	67.4
LEVEL 5	28.40	3	78.6	81.1	80.3	1.4	76.7	83.8
LEVEL 6	44.80	3	95.1	97.5	96.4	1.3	93.3	99.6

----- HOUR=72 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-16.9	19.9	0.0	15.9	-16.6	16.7
LEVEL 1	1.64	3	-8.2	6.9	1.1	8.2	-19.2	21.5
LEVEL 2	3.51	3	-6.0	15.5	4.7	10.8	-22.1	31.6
LEVEL 3	7.41	3	0.4	32.9	15.5	16.4	-25.0	56.1
LEVEL 4	14.80	3	32.9	43.7	36.5	6.3	20.9	52.0
LEVEL 5	28.40	3	81.2	85.5	83.2	2.2	77.9	88.6
LEVEL 6	44.80	3	99.6	100.2	99.9	0.3	99.1	100.8

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ABC LABORATORIES, INC.
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION,
 AND UPPER AND LOWER 95% CONFIDENCE LIMITS)
 BY TREATMENT GROUP

----- HOUR=96 -----

TREATMNT	CONC	NO_REPS	TRT_MIN	TRT_MAX	TRT_MEAN	TRT_SDEV	TRT_LCI	TRT_UCI
CONTROL	0.00	6	-13.4	10.7	0.0	9.2	-9.6	9.7
LEVEL 1	1.64	3	-0.3	10.0	5.0	5.2	-7.9	18.0
LEVEL 2	3.51	3	-2.3	8.7	2.4	5.7	-11.7	16.5
LEVEL 3	7.41	3	-6.9	13.9	3.3	10.4	-22.6	29.2
LEVEL 4	14.80	3	19.1	39.3	30.4	10.3	4.8	56.1
LEVEL 5	28.40	3	84.3	87.6	86.5	1.9	81.8	91.2
LEVEL 6	44.80	3	99.6	100.0	99.9	0.2	99.3	100.4

ABC LABORATORIES, INC. 18
 SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
 ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
 STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN
 ANALYSIS OF NUMBER OF CELLS DIVIDED BY 10**4
 ALL VALUES IN THE DATA FILE ARE BEING PROCESSED
 FITTING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100% INHIBITION
 RESULTS FROM FITTING THE LOGISTIC MODEL
 USING NONLINEAR (WEIGHTED) REGRESSION

FOR HOUR= 96

ESTIMATES OF COEFFICIENTS:
 SLOPE= 4.24 AND EC50= 18.068158468

APPROXIMATE TEST FOR SIGNIFICANCE OF REGRESSION:
 F= 705 P= 0.0000 DEGREES OF FREEDOM=1,16

GOODNESS OF FIT STATISTICS:
 ROOT MEAN SQUARE ERROR (RMSE)= 6.45
 R-SQUARED= 97.80

FOR EACH PERCENT, THE EFFECTIVE CONCENTRATION (EC) AND
 THE LOWER AND UPPER 95% CONFIDENCE LIMITS

PERCENT= 5	EC= 9.0250338621 95% LOWER LIMIT= 8.0245938843 95% UPPER LIMIT= 10.02547384
PERCENT= 10	EC= 10.76346364 95% LOWER LIMIT= 9.7883265753 95% UPPER LIMIT= 11.738600704
PERCENT= 20	EC= 13.031030787 95% LOWER LIMIT= 12.117022391 95% UPPER LIMIT= 13.945039182
PERCENT= 50	EC= 18.068158468 95% LOWER LIMIT= 17.250626222 95% UPPER LIMIT= 18.885690714
PERCENT= 90	EC= 30.330232102 95% LOWER LIMIT= 28.273261399 95% UPPER LIMIT= 32.387202805

ABC LABORATORIES, INC.
SAS PROGRAM AL_ACUTE (VER 2.3) RUN ON 14JUL10
ALGAL TOXICITY TEST FOR TEST MATERIAL: NAPHTHENIC ACIDS
STUDY NUMBER: 64405 --- DATA FILE: U:\REBSTOCKM\SAS\64405YIELD.PRN

THIS COMPLETE ANALYSIS WAS CONDUCTED

BY: MATTHEW REBSTOCK ON: 14JUL10
MAR 14 JUL 10

THE ANALYSIS WAS REVIEWED

BY: AAL ON: 20SEP10