# Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) Spray Dried Powder for Controlling Zebra Mussels Adhering to Test Substrates

By James A. Luoma, Todd J. Severson, Kerry L. Weber, and Denise A. Mayer

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# Conversion Factors

International System of Units to Inch/Pound

| Multiply | By | To obtain |
|---|---|---|
| | Length | |
| centimeter (cm) | 0.3937 | inch (in.) |
| micrometer (µm) | $3.937 \times 10^{-5}$ | inch (in.) |
| millimeter (mm) | 0.03937 | inch (in.) |
| meter (m) | 1.094 | yard (yd) |
| nanometer (nm) | $3.937 \times 10^{-8}$ | inch (in.) |
| | Area | |
| hectare (ha) | 2.471 | acre |
| | Volume | |
| liter (L) | 1.057 | quart (qt) |
| milliliter (mL) | 0.03382 | ounce, fluid (fl. oz) |
| | Flow rate | |
| liter per minute (L/min) | 0.2642 | gallon per minute (gal/min) |
| milliliter per minute (mL/min) | 0.0002642 | gallon per minute (gal/min) |
| | Mass | |
| gram (g) | 0.03527 | ounce, avoirdupois (oz) |
| milligram (mg) | $3.527 \times 10^{-5}$ | ounce, avoirdupois (oz) |

Conductivity is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L).

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.

# Abbreviations

| | |
|---|---|
| AEH | Aquatic Ecosystem Health |
| ASTM | American Society for Testing and Material |
| BI | benthic injection |
| $CaCO_3$ | calcium carbonate |
| d | day(s) |
| DNR | Department of Natural Resources |
| DO | dissolved oxygen |
| EPA | U.S. Environmental Protection Agency |
| h | hour(s) |
| ID | inside diameter |
| MBI | Marrone Bio Innovations |
| $NH_3$ | un-ionized ammonia |
| SAS | Statistical Analysis Software |
| SDP | spray dried powder |
| TAN | total ammonia nitrogen |
| *Pf*-CL145A | *Pseudomonas fluorescens* strain CL145A |
| UMESC | Upper Midwest Environmental Sciences Center |
| USGS | U.S. Geological Survey |
| WWC | whole water column |

# Efficacy of *Pseudomonas fluorescens* (*Pf*–CL145A) Spray Dried Powder for Controlling Zebra Mussels Adhering to Test Substrates

By James A. Luoma,[1] Todd J. Severson,[1] Kerry L. Weber,[1] and Denise A. Mayer[2]

## Abstract

A mobile bioassay trailer was used to assess the efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) formulation for controlling zebra mussels (*Dreissena polymorpha*) from two midwestern lakes: Lake Carlos (Alexandria, Minnesota) and Shawano Lake (Shawano, Wisconsin). The effects of SDP exposure concentration and exposure duration on zebra mussel survival were evaluated along with the evaluation of a benthic injection application technique to reduce the amount of SDP required to induce zebra mortality.

Groups of zebra mussels were collected from each lake and allowed to adhere to test substrates for at least 15 days before exposure to SDP. Two independent trials were completed at each lake: (1) a whole water column (WWC) application trial was used to evaluate the effects of SDP exposure concentration and exposure duration on zebra mussel survival; and (2) a benthic injection (BI) application trial in which the SDP was injected into the test tanks to determine the efficacy of a benthic injection application technique to reduce the amount of SDP required to induced zebra mussel mortality. Three exposure durations (6, 9, and 12 hours) were evaluated in the WWC trials and a 12-hour exposure duration was evaluated in the BI trials. All trials contained zebra mussels which were removed at the completion of each exposure duration, consolidated into wire mesh cages, and held in the lake for approximately 30 days before being assessed for survival.

For all trials, treatment was assigned to each test tank according to a randomized block design ($n$ = 3 test tanks per treatment). The treatment groups included (1) an untreated control group, (2) a group that received an application of 50 milligrams of SDP per liter (mg SDP/L), and (3) a group that received an application of 100 mg SDP/L. During the BI trials, SDP was administered to achieve the desired exposure concentration in the bottom 50 percent (175 L) of the test tank. All exposure concentrations are reported as active ingredient.

Approximately 30 days after exposure, zebra mussels were sorted into live and dead, and enumerated. Mean survival of zebra mussels in control treatments exceeded 95 percent. Mean survival of zebra mussels in the Lake Carlos WWC SDP-treated groups ranged from 0.5 to 2.1 percent and when compared at the same exposure duration, no difference was detected in survival between the 50 and 100 milligrams per liter (mg/L) treatment groups. Similarly, mean survival of zebra mussels in the Shawano Lake WWC SDP-treated groups ranged from 2.0 to 12.6 percent and when compared at the same exposure duration, no difference was detected in survival between the 50- and 100-mg/L treatment

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groups. Mean survival of zebra mussels in the Lake Carlos BI trial SDP-treated groups did not differ ($p = 0.93$) and was 18.1 and 18.0 percent in the 50- and 100-mg/L treatment groups, respectively. Mean survival of zebra mussels in the Shawano Lake BI trial SDP-treated groups differed ($p < 0.01$) and was 2.9 and 0.9 percent in the 50- and 100-mg/L treatment groups, respectively. Survival of zebra mussels assigned to the SDP-treated groups in the Lake Carlos WWC trial (12-hour exposure duration) differed from the survival of zebra mussels assigned to the SDP-treated groups in the Lake Carlos BI trial; however, after modification of the BI application technique, no difference ($p = 0.22$) was detected between the survival of zebra mussel in the Shawano Lake WWC (12-hour exposure duration) and BI trials.

## Introduction

Freshwater mussels native to North America (order Unionoida) are considered the most diverse in the world, consisting of approximately 297 recognized taxa, but they have been declining at an alarming rate due to anthropogenic activities such as pollution, habitat alteration, and over harvest (Williams and others, 1993; Neves and others, 1997). A survey completed by the Nature Conservancy revealed 55 percent of North America's mussels are either extinct or imperiled (Master, 1990). The North American mussel extinction rate is predicted to be 6.4 percent per decade, which equates to the extinction of 127 species in the next 100 years (Ricciardi and Rasmussen, 1999). This prediction may be conservative, as it did not account for the invasion of North American waterways by dreissenid mussels (zebra mussel, *Dreissena polymorpha* and quagga mussel, *Dreissena bugensis*).

Dreissenid mussels are ideal invaders due to their high fecundity and their planktonic larvae, which are capable of dispersal over vast areas (Birnbaum, 2011). Dreissenid mussels pose a serious ecological threat and negatively affect many native aquatic species, particularly freshwater mussels. Dreissenid mussels can quickly inundate freshwater mussels and cause obstruction of valve movement as well as inhibition of feeding and respiration (Burlakova and others, 2000) resulting in an increased cost of metabolism, decreased fitness, and ultimately death (Baker and Hornbach, 1997).

Natural resource managers lack readily available, environmentally safe, and effective tools for controlling dreissenid mussels in open-water environments. One potential tool for limited open-water control of dreissenid mussels is a commercially formulated spray dried powder (SDP) formulation of *Pseudomonas fluorescens* (Zequanox®), produced by Marrone Bio Innovations, Inc. (MBI; Davis, California), which contains nonviable cells of a specific strain (CL145A) of the common soil bacterium *Pseudomonas fluorescens*. The SDP formulation was developed by MBI and registered by the U.S. Environmental Protection Agency for control non-native dreissenid (zebra and quagga) mussels in raw water conduit systems (that is, industrial cooling and irrigation systems, and so forth) and it has recently been approved for use in limited open-water environments.

The objectives of this study were (1) to evaluate the potential use of *Pseudomonas fluorescens* spray dried powder (SDP) formulation for controlling zebra mussels (*Dreissena polymorpha*) in limited open-water environments; and (2) to evaluate the use of a benthic injection (BI) application technique to reduce the amount of SDP required to induce zebra mussel mortality.

The applications for this study were completed in the Upper Midwest Environmental Science Center's (UMESC) mobile bioassay laboratory, which used water and test animals from two midwestern lakes: Lake Carlos (Alexandria, Minnesota) and Shawano Lake (Shawano, Wisconsin). This final study report summarizes four separate field trials with activities carried out from October, 2011 to November, 2013. Applications of SDP were completed on August 15 and 17, 2012 at Lake Carlos and on September 6 and 8, 2012 at Shawano Lake.

# Materials and Methods

All methods and materials followed the written protocol and its amendments, except those instances that were identified as deviations (appendix 2, items 1–6). The study protocol and amendments for this study are contained in appendix 1 (items 1–2).

## Experimental Design

The study SDP applications were completed within the UMESC mobile bioassay laboratory, which used water and test animals from two midwestern lakes: Lake Carlos (Alexandria, Minn.) and Shawano Lake (Shawano, Wis.). Survival of zebra mussels was assessed approximately 30 days after exposure to SDP. Groups of zebra mussels were collected from each lake and allowed to adhere to perforated aluminum test substrates for at least 15 days before exposure to SDP. Zebra mussels adhering to the test substrates were exposed to SDP in a series of nine 350-liter (L) test. Two independent trials were completed at each test location (1) a whole water column (WWC) application trial, which evaluated the effects of SDP exposure concentration and exposure duration on zebra mussel survival, and (2) a BI application trial, which evaluated the use of a BI application technique to reduce the amount of SDP required to induce zebra mussel mortality.

Treatments were administered in triplicate according to a randomized block design (appendix 3, items 1, 4, 7, and 10) and included (1) an untreated control group, (2) a group that received an application of 50 milligrams SDP per liter (mg SDP/L), and (3) a group that received an application of 100 mg SDP/L. The experimental unit was the individual test tank. Test substrates with adhering zebra mussels were distributed to test tanks according to a random distribution scheme (appendix 3, items 2, 5, 8, and 11).

Each WWC test tank contained nine test substrates with adhering zebra mussels and each BI test tank contained either three (Lake Carlos) or four (Shawano Lake) test substrates with adhering zebra mussels. Upon exposure termination during the WWC trials (6, 9, and 12 hours), three randomly selected test substrates were removed from each test tank. Upon exposure termination during the BI trials (12 hours), all test substrates were removed from each test tank. After exposure, the test substrates with adhering zebra mussels were consolidated into wire mesh cages, which were placed in approximately 2.5 meters (m) of water for the post-exposure period. Approximately 30 days after SDP exposure, zebra mussels were sorted into live and dead, and enumerated. Zebra mussels from one test substrate of each treatment level and exposure duration were retained in 70 percent isopropyl alcohol for length measurement.

## Test Article

The test article was a commercially prepared SDP formulation of *Pseudomonas fluorescens*, strain CL145A containing 50 percent active ingredient (weight to weight ratio [w/w] *P. fluorescens*, strain CL145A). The test article was provided by the manufacturer as a mixed lot (401P12163C and 401P12164C; Certificates of Analysis, appendix 4, items 3 and 4). Test article use was documented in the test chemical log books (appendix 4, items 9–13). Concentrations of the test article are reported as active ingredient. Retention of test article biological activity was assessed after exposure by New York State Museum Field Research Laboratory (Cambridge, New York) using their standard dreissenid mussel bioassay (appendix 4, item 8). Results of the biological activity bioassay demonstrated a mean (standard deviation) mortality of 70.7 percent (4.6) at 200 mg/L, which was similar to mean mortality exhibited by the cell fraction positive control which was 73.3 percent (8.3), confirming the biological activity of the test article.

## Test Locations

Two midwestern lakes with different water-quality characteristics were the test locations and the source of the test water and test animals. Lake Carlos is a 1,020-hectare (ha) mesotrophic lake located near Alexandria, Minn., and it is the deepest natural lake in Minnesota (excluding Lake Superior), with a maximum depth of 49.7 m. Shawano Lake is a 2,515-ha eutrophic lake located in Shawano, Wis., with a maximum depth of 12 m. Zebra mussels were first reported in Shawano Lake in 2002 and in Lake Carlos in 2009 (Turyk and others, 2008; Engel and others, 2010).

## Test System

The test system was a series of nine independent circular test tanks (76 x 95 centimeters (cm), diameter x height; 350 L capacity) contained within the UMESC mobile bioassay laboratory. The test tanks were positioned in two rows with four test tanks in one row and five test tanks in the other (fig. 1). Test substrates were used as the medium to facilitate zebra mussel handling during the study period. The test substrates were constructed of perforated aluminum (4.8 millimeter (mm) hole, 51 percent open area, 1.6 mm thick) folded into trays (15.2 x 15.2 x 2.5 cm, length x width x height [fig. 2]).

Test water was supplied to the test system from a 3-horsepower submersible well pump (ITT Goulds Pumps, Seneca Falls, N.Y., model 18GS30). The water was filtered (200 micrometers [µm]) using a microscreen filtration system (Forstra Filter Inc., Los Angeles, California; model M1-90), delivered to two headboxes (30.5 x 55.9 x 114.3 x 30.5 cm, width x length x height; one headbox per test tank row), and gravity fed to each test tank at approximately 6 liters per minute, providing approximately one tank-exchange per hour. Water flow was interrupted during the exposure period. Untreated water was discharged to the lake; SDP-treated water was collected in frame tanks and removed by a state-licensed septic hauler and disposed of by land application (Minnesota) or discharge to a sanitary sewer system (Wisconsin).

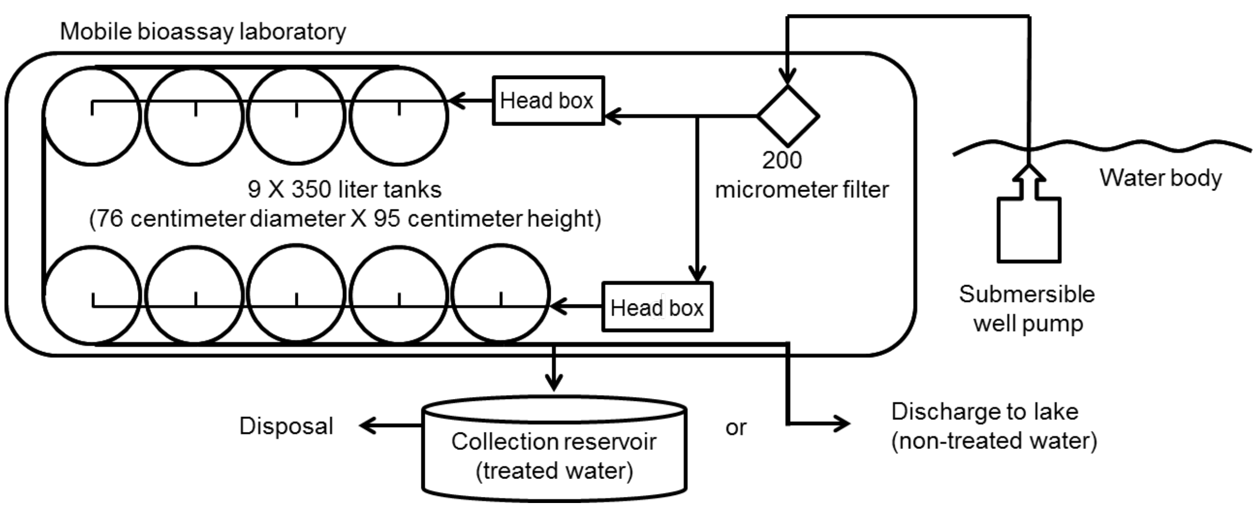


Figure 1. Schematic of mobile bioassay laboratory.

Figure 2. Example of test substrate with zebra mussels during initial placement (left), after adherence (center), and prepared for exposure in a semirigid plastic mesh containment bag (right).

## Test Animals

Zebra mussels were collected from existing colonies within each lake and placed on test substrates. Zebra mussels adhering to the test substrates immediately prior to allocation to the test tanks were used as the test animals. Mean shell length for the zebra mussels used in the Lake Carlos trials ranged from 11.26 to 11.85 mm and the mean shell length for the zebra mussels used in the Shawano Lake trials ranged from 18.27 to 18.74 mm (appendix 5, items 2–5).

### Test Animal Collection and Initial Placement

In October and November 2011, natural substrates (that is, rocks, sticks, and native mussels) with adhering zebra mussels were collected from each lake and the zebra mussels were removed by severing the byssus with a scalpel. Zebra mussels were held in coolers containing lake water until placed onto the test substrates. Approximately 200 to 300 zebra mussels were indiscriminately selected and placed on each test substrate. After zebra mussel placement, wood spacers (≈ 2 cm thick) were used to separate the test substrates (fig. 3) before they were secured in vertical stacks (≈ 10 substrates per stack). Six stacks were placed in each of three wire mesh cages at each lake (≈ 180 substrates per lake; fig. 3). The wire mesh cages were placed in ≈ 2 m of water to allow for zebra mussel adherence through winter. Due to poor overwinter survival, zebra mussels on the Shawano Lake test substrates were replaced in August of 2012 following the procedures previously described. The zebra mussels used in the Shawano Lake trial were allowed to adhere to the test substrates ≈ 16 days prior to exposure.

Figure 3. Example of stacked test substrates (left) and wire mesh cage (right).

### Preparation and Distribution to the Test System

One day prior to SDP exposure, test substrates were inverted to dislodge non-adhering zebra mussels. Moribund zebra mussels and shell fragments were removed from each test substrate with forceps. Test substrates with adhering zebra mussels were placed into a uniquely identified semirigid plastic mesh containment bags (20.3 x 25.4 x 5.1 cm; 0.32 x 0.42 cm openings) and randomly allocated to test tanks (appendix 3, items 2, 5, 8, and 11). The WWC trials at both lakes received nine test substrates per test tank (three per exposure duration), whereas the BI trials received three (Lake Carlos) or four (Shawano Lake) test substrates per test tank.

### Post-Exposure Handling

Upon exposure termination (that is, 6, 9, and 12 hours [h] for WWC; 12 h for BI), test substrates were removed from the test tanks according to a randomization scheme (appendix 3, items 3, 6, 9, and 12) and consolidated in wire mesh cages. Test substrates removed from all test tanks at each exposure duration were indiscriminately placed into a single wire mesh cage (for example, all test substrates removed at 6 h were placed into the same wire mesh cage). The wire mesh cages were placed in ≈ 2.5 m of water, in the respective lake, for the ≈ 30-day post-exposure period.

### Survival Assessment

Zebra mussel survival was assessed 26–27 days after SDP exposure at Lake Carlos and 32–34 days after SDP exposure at Shawano Lake. Survival was determined by examining valve movement in response to mechanical stimuli or resistance to valve pressure by adductor muscle contraction. Zebra mussels < 6 mm were excluded from the analyses because they could readily pass through the plastic mesh containment bags and because of the potential for inconsistent survival assessment of small mussels (that is, inconsistent assessment of adductor muscle response). Zebra mussels from each test substrate were sorted into groups of live or dead and enumerated. Zebra mussels from one test substrate at each treatment level and exposure duration were indiscriminately selected and retained in 70 percent isopropyl alcohol to measure for shell length with digital calipers.

## Dosing

Stock solutions used to administer treatments were prepared by adding pre-weighed aliquots of SDP (appendix 4, items 5 and 6) into known volumes of filtered (200 µm) lake water and mechanically mixing with a paint mixer attached to an electric drill for 3–5 minutes. The solution was then immediately poured through a mesh colander and any clumps of SDP were pulverized with a pestle and rinsed into the stock solution with filtered lake water. During all trials, control treatments were completed using the same methods as the SDP-treated groups with the exception that no SDP was applied. For all trials, concentrations of SDP are reported as active ingredient.

### Whole Water Column Application

Separate stock solutions were prepared for each test tank replicate in the WWC trials. Stock solutions for the WWC were prepared by adding SDP (35 grams [g] for the 50 milligrams per liter [mg/L] treatment; 70 g for the 100-mg/L treatment) to ≈ 8 L of water removed from each test tank. Immediately after preparation, each stock was poured into the respective test tank and gently mixed with the test tank water.

## Benthic Injection Application

An injection system was constructed to treat the bottom 50 percent (175 L) of each test tank during the BI trials (fig. 4). A peristaltic pump (Masterflex Digi-staltic drive, model 77310; Cole-Parmer, Vernon Hills, Illinois) was used to inject the SDP stock at the selected depth in each test tank through a delivery apparatus consisting of four peristaltic tubing lines (Masterflex L/S 14 tubing; 1.6 mm Inside Diameter [ID]), which terminated with dispersion nozzles designed to disperse test article horizontally in the water column. Each dispersion nozzle was constructed from a modified plastic cylindrical check valve (Penn-Plax, Inc.; model CV1 check-valve/air-filter; Hauppauge, N.Y.) with four 2-mm holes drilled around the circumference (fig. 4). At Shawano Lake, the SDP stock concentrations were diluted, the stock delivery rate was increased, and the injection height was raised to decrease SDP settling.

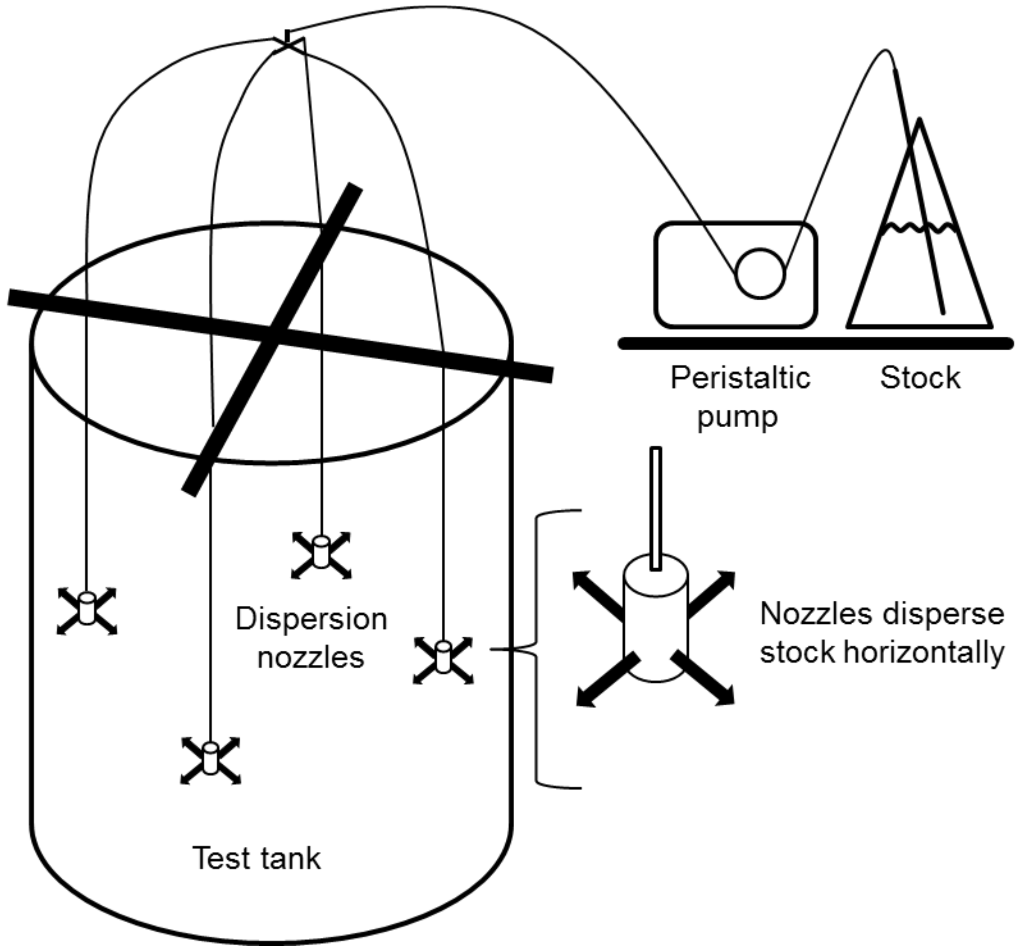


Figure 4. Schematic of benthic injection application system.

### Benthic Injection Application at Lake Carlos

Separate 15,000 and 30,000 mg SDP/L stock solutions were prepared for the 50- and 100-mg/L Lake Carlos BI treatment applications, respectively. Approximately 583 milliliters (mL) of the appropriate stock solution was delivered to each test tank at 62 mL/minute, resulting in delivery of ≈ 8,750 and 17,500 mg of SDP to each 50 and 100 mg/L test tank replicate, respectively. The amount of stock solution injected was the volume required to achieve the target SDP exposure concentration in the bottom 50 percent (175 L) of the test tank. The stock solution was delivered ≈ 19 cm from the bottom of each test tank.

### Benthic Injection Application at Shawano Lake

Separate 2,500 and 5,000 mg SDP/L stock solutions were prepared for the 50- and 100-mg/L Shawano Lake BI treatment applications, respectively. Approximately 3,500 mL of the appropriate stock solution was delivered to each test tank at 350 mL/minute, resulting in delivery of ≈ 8,750 and 17,500 mg of SDP to each 50 and 100 mg/L test tank replicate, respectively. The amount of stock solution injected was the volume required to achieve the target SDP exposure concentration in the bottom 50 percent (175 L) of the test tank. The stock solution was delivered ≈ 38 cm from the bottom of each test tank.

## Exposure Concentrations

Water samples were collected for SDP exposure concentration determination from different locations within test tanks depending on application type (WWC or BI) and trial location. Surface samples were collected by submersing a 50-mL beaker below the water surface. Suspended (≈ 15 or 19 cm from the test tank bottom) and bottom samples (Lake Carlos BI trial only) were collected from test tanks using a peristaltic pump (Masterflex Digi-staltic drive, model 77310; Cole-Parmer, Vernon Hills, Ill.) fitted with four 1.6-mm ID collection lines. Approximately 200 mL of exposure water was purged through peristaltic tubing and discarded before sample collection.

Exposure concentrations were determined by comparing the test tank water sample absorbance to a linear regression curve created from known active ingredient concentrations of SDP (25, 50, 100, and 200 mg/L). Absorbance was measured using a Barnstead/Turner SP-830 Plus spectrophotometer (model SM110215) at 660 nanometers (nm). Linear regression equations were fit using the Statistical Analysis Software Proc Reg procedure (SAS® Version 9.3, SAS Institute, Inc., Cary, North Carolina). The exposure concentrations were predicted from the regression analysis (appendix 7, items 2–4, 6–8, 10–12, 14–16). The known concentrations of SDP used to create the linear regression were maintained at approximately 4 degrees Celsius (ºC) and measured for absorbance at 6, 9, and 12 h after treatment administration to ensure proper spectrophotometer function with the exception of the Lake Carlos WWC trial, in which the known concentrations were measured at 9 and 12 h.

The SDP exposure concentrations in the Lake Carlos WWC trial were determined from surface water samples at 1, 3, 6, 9, and 12 h and suspended (≈ 15 cm) water samples at 3, 6, 9, and 12 h. The SDP exposure concentrations in the Lake Carlos BI trial were determined from suspended (≈ 19 cm) water samples at 1, 3, 6, 9, and 12 h and from bottom water samples at 6, 9, and 12 h. Because sample absorbance was below the detection limit, samples from control test tanks were not analyzed for SDP concentration during the Lake Carlos trials. Control water samples were analyzed for SDP in the Shawano Lake trials, and were below the detection limit. The SDP exposure concentrations in the Shawano Lake WWC trial were determined from surface water and suspended (≈ 15 cm) water samples at 1, 3, 6, 9, and 12 h. The SDP exposure concentrations in the Shawano Lake BI trial were determined from surface water and suspended (≈ 15 cm) water samples at 1, 3, 6, 9, and 12 h.

## Water Chemistry

Dissolved oxygen, pH, temperature, hardness, alkalinity, and conductivity were measured ≈ 1 h prior to treatment administration from filtered lake water samples collected from the distribution headboxes. Dissolved oxygen, pH, and temperature were measured in each test tank within 1 h and ≈ 3, 6, 9, and 12 h after treatment administration during the WWC and BI trials.

Water samples for total ammonia nitrogen (TAN) analysis were collected 12 h after SDP application during all trials and at 6 and 9 h after SDP application during the Lake Carlos WWC trial. Water samples were filtered (0.45 µm), acidified with 10 percent sulfuric acid to ≤ pH 2.5, and stored at ≈ 4 ºC until analyzed for TAN by the UMESC water-quality laboratory using the automated phenate method (Standard Method 4500G; American Public Health Association, 2012). Un-ionized ammonia concentrations were calculated from TAN, pH, and temperature from time of sample collection using the formula identified by Emerson and others (1975).

Four data loggers (Onset Inc, Bourne, Massachusetts; HOBO® Pendent Temperature/Light Data Logger, model UA-002064) were attached to the wire mesh cages at each lake and used to measure water temperature four times daily during the post-exposure period.

## Data Analysis

Data analyses for water chemistry parameters were limited to simple summary statistics; comparative statistics were not generated. Exposure concentration means were determined using SAS® software version 9.3 (SAS Institute, Inc., Cary, N.C.). The SAS® software Proc Means procedure was used to determine the mean exposure concentration by individual test tank, treatment group, and exposure duration (appendix 7, items 2–4, 6–8, 10–12, 14–16). Because of observations of SDP settling and non-detectable levels of SDP in the suspended water samples, the bottom water sample exposure concentrations for the Lake Carlos BI trial are reported. The suspended water exposure concentrations for Shawano Lake BI trials are reported due to negligible (≤ 3mg/L) SDP exposure concentrations measured in the surface water samples.

### Zebra Mussel Survival

Statistical comparisons of zebra mussel survival were completed using SAS® software version 9.3. Significance was declared at $\alpha \leq 0.05$. A generalized linear mixed model was used to analyze the survival of zebra mussels in each treatment group (appendix 9, items 1–3). The proportion of mortalities (number of dead zebra mussels compared to the total number of zebra mussels in the sample) was modeled using the SAS® software Proc GLIMMIX procedure with a binomial distribution and a logit link function. A scale parameter was added to the model using the SAS® software random_residual_ statement. Zebra mussel survival in each treatment group was individually compared to the zebra mussel survival in the untreated control group using a two-sided means comparison test.

## Results

Water chemistry parameters (dissolved oxygen, pH, and temperature) collected from the test tanks are summarized in table 1 and the water chemistry data are in appendix 6 (items 1–14). Water hardness, alkalinity, and conductivity are summarized in table 2. Dissolved oxygen levels remained above the minimum threshold recommended for freshwater mussels (4.0 mg/L, ASTM International, 2013) and the mean TAN remained below the criterion for acute exposure and below 4-day maximum criterion for chronic exposure during the exposure period (U.S. Environmental Protection Agency, 2013; table 3). Mean daily temperatures ranged from 20.6 to 24.4 °C (Lake Carlos) and from 10.1 to 21.5 °C (Shawano Lake) during the post-exposure period.

**Table 1.** Mean (standard deviation) water chemistry parameters (dissolved oxygen and temperature) and pH range of each treatment group during the study period.

[mg/L, milligrams per liter; DO, dissolved oxygen; °C, degrees Celsius]

| Water chemistry parameter | Treatment group (mg/L) | Pre-exposure[1] | ≤1 h | 3 h | 6 h | 9 h | 12 h |
|---|---|---|---|---|---|---|---|
| Lake Carlos whole water column application | | | | | | | |
| DO (mg/L) | 0 | 8.77 (0.02) | 8.72 (0.04) | 8.58 (0.09) | 8.51 (0.04) | 8.25 (0.03) | 8.11 (0.01) |
| | 50 | 8.76 (0.01) | 8.66 (0.02) | 8.42 (0.01) | 8.21 (0.04) | 7.80 (0.09) | 6.62 (0.13) |
| | 100 | 8.76 (0.01) | 8.64 (0.01) | 8.38 (0.04) | 8.06 (0.10) | 7.74 (0.07) | 6.84 (0.07) |
| pH | 0 | 8.62–8.64 | 8.60–8.63 | 8.56–8.58 | 8.54–8.58 | 8.25–8.31 | 8.55–8.58 |
| | 50 | 8.61–8.65 | 8.57 | 8.47–8.49 | 8.47–8.49 | 8.20–8.21 | 8.33–8.35 |
| | 100 | 8.60–8.63 | 8.53 | 8.37–8.39 | 8.37–8.39 | 8.12 | 8.21–8.24 |
| Temperature (°C) | 0 | 22.2 (<0.1) | 22.3 (<0.1) | 22.4 (<0.1) | 22.5 (<0.1) | 22.5 (<0.1) | 22.5 (<0.1) |
| | 50 | 22.1 (<0.1) | 22.3 (<0.1) | 22.4 (<0.1) | 22.5 (0.0) | 22.4 (0.1) | 22.4 (<0.1) |
| | 100 | 22.2 (0.0) | 22.3 (0.0) | 22.4 (<0.1) | 22.5 (<0.1) | 22.4 (0.1) | 22.4 (0.1) |
| Shawano Lake whole water column application | | | | | | | |
| DO (mg/L) | 0 | 7.34 (0.02) | 7.24 (0.07) | 7.07 (0.03) | 6.85 (0.06) | 6.63 (0.02) | 6.43 (0.10) |
| | 50 | 7.36 (0.02) | 7.24 (0.04) | 6.97 (0.10) | 6.57 (0.08) | 5.86 (0.19) | 4.22 (0.64) |
| | 100 | 7.36 (0.01) | 7.25 (0.07) | 7.01 (0.03) | 6.56 (0.08) | 6.03 (0.18) | 4.79 (0.55) |
| pH | 0 | 9.31–9.34 | 9.27–9.28 | 9.23–9.24 | 9.14–9.15 | 9.12–9.16 | 9.02–9.08 |
| | 50 | 9.33–9.34 | 9.22 | 9.11–9.14 | 9.06–9.08 | 8.99–9.02 | 8.73–8.85 |
| | 100 | 9.34 | 9.14–9.16 | 9.07–9.08 | 8.97–9.00 | 8.87–8.93 | 8.62–8.73 |
| Temperature (°C) | 0 | 22.6 (0.0) | 22.0 (<0.1) | 22.0 (0.2) | 22.1 (0.3) | 22.2 (0.4) | 22.0 (0.4) |
| | 50 | 22.6 (<0.1) | 21.9 (0.1) | 21.9 (0.1) | 21.9 (0.2) | 21.9 (0.2) | 21.8 (0.2) |
| | 100 | 22.6 (0.0) | 21.9 (<0.1) | 21.9 (0.1) | 22.0 (0.1) | 22.0 (0.1) | 21.8 (0.1) |
| Lake Carlos benthic injection application | | | | | | | |
| DO (mg/L) | 0 | 8.42 (0.01) | 7.97 (0.02) | 7.97 (0.02) | 7.95 (0.03) | 7.84 (0.05) | 7.79 (0.05) |
| | 50 | 8.40 (0.02) | 7.97 (0.02) | 7.97 (0.02) | 7.83 (0.05) | 7.81 (0.01) | 7.16 (0.26) |
| | 100 | 8.40 (0.03) | 7.90 (0.08) | 7.95 (0.02) | 7.91 (0.02) | 7.81 (0.05) | 7.24 (0.14) |
| pH | 0 | 8.70 | 8.52–8.59 | 8.51–8.55 | 8.47–8.50 | 8.38–8.39 | 8.55–8.58 |
| | 50 | 8.70 | 8.60–8.61 | 8.55 | 8.48 | 8.38–8.41 | 8.12–8.20 |
| | 100 | 8.69–8.70 | 8.57–8.60 | 8.52–8.55 | 8.44–8.48 | 8.39–8.41 | 7.18–7.39 |
| Temperature (°C) | 0 | 21.2 (0.0) | 21.3 (0.0) | 21.2 (0.0) | 21.5 (0.1) | 21.4 (0.2) | 21.0 (0.1) |
| | 50 | 21.2 (<0.1) | 21.3 (0.0) | 21.2 (<0.1) | 21.7 (0.3) | 21.6 (0.3) | 21.1 (0.2) |
| | 100 | 21.2 (0.0) | 21.3 (0.1) | 21.3 (<0.1) | 21.6 (0.1) | 21.5 (0.1) | 21.1 (0.1) |

| Water chemistry parameter | Treatment group (mg/L) | Pre-exposure[1] | ≤1 h | 3 h | 6 h | 9 h | 12 h |
|---|---|---|---|---|---|---|---|
| | | Shawano Lake benthic injection application | | | | | |
| DO (mg/L) | 0 | 7.41 (0.01) | 7.26 (0.01) | 7.15 (0.07) | 7.02 (0.01) | 6.98 (0.04) | 6.63 (0.02) |
| | 50 | 7.43 (<0.1) | 7.22 (0.03) | 7.23 (0.01) | 7.28 (0.02) | 7.14 (0.07) | 5.11 (0.67) |
| | 100 | 7.44 (<0.1) | 7.17 (0.03) | 7.23 (0.03) | 7.29 (0.03) | 7.24 (0.01) | 6.21 (0.34) |
| pH | 0 | 9.10–9.12 | 9.05–9.06 | 9.02–9.03 | 8.91–8.94 | 8.64–8.68 | 8.92–8.94 |
| | 50 | 9.13–9.14 | 9.02–9.04 | 9.02–9.04 | 8.93–8.95 | 8.69–8.71 | 8.69–8.88 |
| | 100 | 9.13–9.14 | 8.99–9.01 | 9.03 | 8.95–8.96 | 8.69–8.71 | 8.58–8.68 |
| Temperature (°C) | 0 | 19.5 (0.0) | 18.2 (0.1) | 18.4 (0.3) | 18.6 (0.3) | 18.5 (0.2) | 18.2 (0.2) |
| | 50 | 19.5 (0.0) | 18.2 (<0.1) | 18.4 (0.1) | 18.5 (0.2) | 18.4 (0.2) | 18.2 (0.1) |
| | 100 | 19.5 (0.0) | 18.0 (0.1) | 18.1 (0.1) | 18.2 (0.1) | 18.2 (0.1) | 17.9 (0.1) |

[1]Pre-exposure time points were measured approximately 1 h prior to test article application.

**Table 2.** Mean (standard deviation) hardness, alkalinity, and conductivity of filtered (200 micrometers) source water collected from the delivery system headboxes prior to exposure.

[mg/L, milligrams per liter; µS/cm, microsiemens per centimeter; <, less-than; CaCO3, calcium carbonate; °C, degrees Celsius]

| Application type | Hardness (mg/L)[1] | Alkalinity (mg/L)[1] | Conductivity (µS/cm)[2] |
|---|---|---|---|
| | Lake Carlos | | |
| Whole water column | 177 (1) | 163 (1) | 395 (1) |
| Benthic injection | 177 (1) | 164 (<1) | 363 (3) |
| | Shawano Lake | | |
| Whole water column | 118 (1) | 105 (<1) | 248 (2) |
| Benthic injection | 125 (1) | 112 (1) | 231 (2) |

[1]Reported as milligrams per liter $CaCO_3$.

[2]Temperature compensated to 25 °C.

**Table 3.** Mean (standard deviation) total ammonia nitrogen (TAN) and un-ionized ammonia ($NH_3$) of each treatment group by lake, application type, and exposure duration.

[mg/L, milligrams per liter; h, hours; WWC, whole water column application; BI, benthic injection application; TAN, total ammonia nitrogen; SD, standard deviation; $NH_3$, un-ionized ammonia; mg $NH_3$-N/L, milligrams un-ionized ammonia nitrogen per liter; -, no sample]

| Water chemistry parameter | Treatment group (mg/L) | 6 h WWC | 9 h WWC | 12 h WWC | 12 h BI |
|---|---|---|---|---|---|
| Lake Carlos | | | | | |
| TAN[1] (SD) | 0 | 0.17 (<0.01) | 0.17 (<0.01) | 0.24 (0.01) | 0.22 (0.01) |
| | 50 | 0.22 (0.01) | 0.23 (<0.01) | 0.33 (0.01) | 0.40 (0.01) |
| | 100 | 0.27 (<0.01) | 0.27 (0.00) | 0.34 (0.01) | 1.37 (0.18) |
| $NH_3$ (SD) | 0 | 0.03 (<0.01) | 0.01 (<0.01) | 0.04 (<0.01) | 0.03 (<0.01) |
| | 50 | 0.03 (<0.01) | 0.02 (0.00) | 0.03 (<0.01) | 0.02 (<0.01) |
| | 100 | 0.03 (<0.01) | 0.02 (0.00) | 0.03 (0.00) | 0.01 (<0.01) |
| Shawano Lake | | | | | |
| TAN[1] (SD) | 0 | - | - | 0.13 (<0.01) | 0.06 (0.01) |
| | 50 | - | - | 0.19 (0.01) | 0.11 (0.01) |
| | 100 | - | - | 0.23 (0.01) | 0.16 (0.01) |
| $NH_3$ (SD) | 0 | - | - | 0.04 (<0.01) | 0.01 (<0.01) |
| | 50 | - | - | 0.04 (<0.01) | 0.02 (<0.01) |
| | 100 | - | - | 0.04 (<0.01) | 0.02 (<0.01) |

[1]Total ammonia nitrogen reported as mg $NH_3$-N/L.

Mean exposure concentrations during the exposure period are shown in figure 5. The SAS® software linear regression, SAS® software predicted exposure concentrations, and data are in appendix 7 (items 1–16). In the Lake Carlos and Shawano Lake WWC trials, the surface water exposure concentrations are reported due to negligible differences between the measured exposure concentrations in the surface water and suspended water samples (mean difference ≤ 0.46 and 0.93 mg/L for the 50- and 100-mg/L treatment groups, respectively). Mean test tank SDP exposure concentrations in surface water samples during the Lake Carlos WWC trial (that is, 6-, 9-, and 12-h exposure durations) ranged from 43.9 to 47.3 mg/L and 90.2 to 95.3 mg/L in the 50- and 100-mg/L treatment groups, respectively (fig. 5). Mean test tank SDP concentrations in surface water samples during the Shawano Lake WWC trial (that is, 6-, 9-, and 12-h exposure durations) ranged from 43.8 to 45.9 mg/L and 93.8 to 99.3 mg/L in the 50- and 100-mg/L treatment groups, respectively.

The SDP concentrations were below the detection limit in the initial suspended samples collected during the Lake Carlos BI trial; therefore, bottom sampling was initiated at 6 hours and continued throughout the duration of the exposure period. The mean SDP concentrations in the bottom samples were 100.3 mg/L for the 50-mg/L treatment group and 234.7 mg/L for the 100-mg/L treatment group. Mean SDP concentrations in suspended samples collected during the Shawano Lake BI trial were 38.8 mg/L for the 50-mg/L treatment group and 92.9 mg/L for the 100-mg/L treatment group.

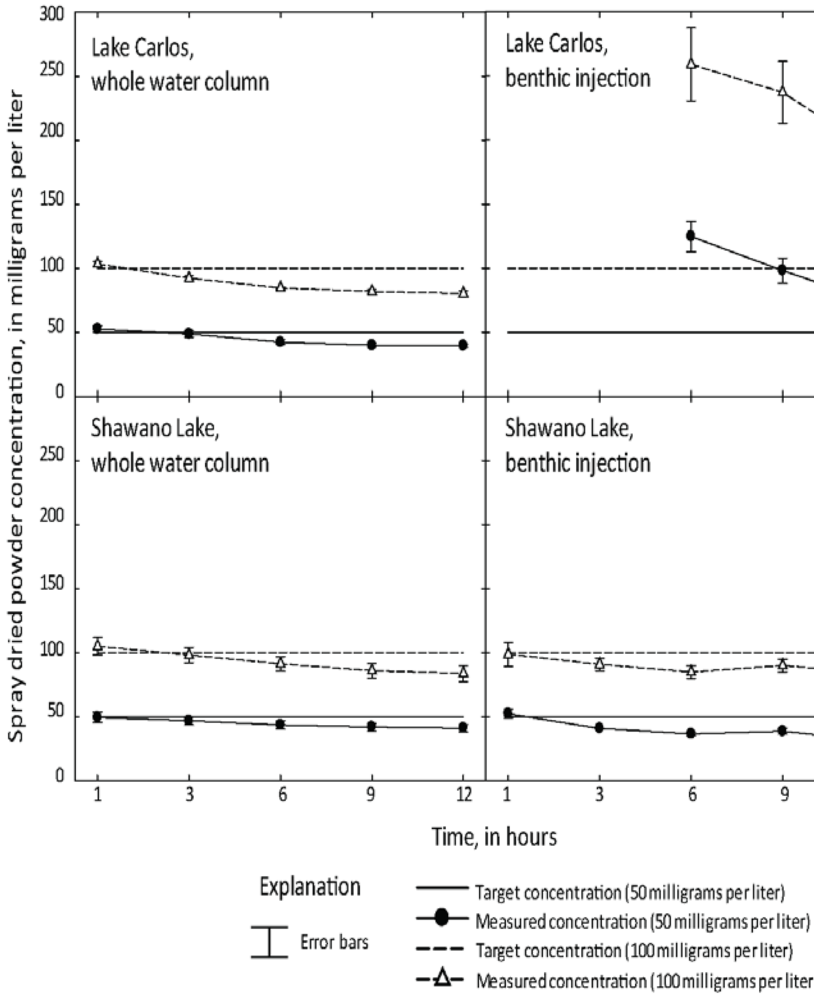


Figure 5. Mean (standard deviation) SDP active ingredient concentration of water samples collected during the exposure period. WWC graphs are from the surface samples; BI graphs are from bottom samples (Lake Carlos) and suspended samples (Shawano Lake)

## Zebra Mussel Survival

Zebra mussel survival for all trials is summarized in table 4, statistical analyses are in appendix 9 (items 1–3), and survival data are in appendix 8 (items 1–5). For all trials (that is, WWC at 6, 9, and 12 h; BI at 12 h), mean survival of control groups exceeded 95 percent and zebra mussel survival in the SDP-treated groups differed ($p < 0.01$) from survival in the control groups.

**Table 4.** Mean (standard deviation) percent zebra mussel survival for each lake, application type, and exposure duration.

[Means within columns and rows (for each lake) with the same letter are not significantly different ($p > 0.05$); means were compared by concentration (a-c), exposure duration (m,n) and between whole water column 12 h exposure duration and benthic injection 12 h exposure duration (y,z); mg/L, milligrams per liter; h, hours; WWC, whole water column application; BI, benthic injection application]

| Test location | Treatment group (mg/L) | 6 h | 9 h | 12 h | 12 h |
|---|---|---|---|---|---|
| | | WWC | | | BI |
| Lake Carlos | 0 | 97.8$^{am}$ (2.2) | 96.9$^{am}$ (1.8) | 97.3$^{amz}$ (1.0) | 97.3$^{az}$ (1.4) |
| | 50 | 1.3$^{bm}$ (1.0) | 1.1$^{bm}$ (1.5) | 0.6$^{bmy}$ (0.6) | 18.1$^{bz}$ (7.3) |
| | 100 | 2.1$^{bm}$ (1.2) | 0.5$^{bn}$ (0.7) | 0.6$^{bny}$ (0.7) | 18.0$^{bz}$ (10.4) |
| Shawano Lake | 0 | 95.6$^{am}$ (2.4) | 95.5$^{am}$ (1.4) | 96.2$^{amz}$ (1.3) | 95.5$^{az}$ (2.3) |
| | 50 | 12.6$^{bm}$ (10.7) | 10.3$^{bm}$ (9.4) | 2.7$^{bnz}$ (2.8) | 2.9$^{bz}$ (1.6) |
| | 100 | 7.2$^{bm}$ (6.6) | 4.7$^{bmn}$ (5.5) | 2.0$^{bnz}$ (1.9) | 0.9$^{cz}$ (1.0) |

## Lake Carlos Whole Water Column Trial

Mean survival of zebra mussels in the Lake Carlos WWC SDP-treated groups ranged from 0.5 to 2.1 percent. When compared at the same exposure duration, no difference in zebra mussel survival was detected between the 50- and 100-mg/L treatment groups. When comparing the effects of exposure duration by treatment group, the only difference in zebra mussel survival detected was in the 100-mg/L treatment group, when the 6-h exposure duration group was compared to the 9-h and 12-h exposure duration groups ($p \leq 0.01$).

## Shawano Lake Whole Water Column Trial

Mean survival of zebra mussels in the Shawano Lake WWC SDP-treated groups ranged from 2.0 to 12.6 percent. When compared at the same exposure duration, no difference ($p > 0.11$) in zebra mussel survival was detected between the 50- and 100-mg/L treatment groups. When comparing the effects of exposure duration by treatment group, no difference was detected in control group survival and in the 50-mg/L treatment group, no difference was detected when comparing the 6-h exposure duration to the 9-h exposure duration ($p = 0.31$). Differences were detected in the 50-mg/L treatment group when the 6 and 9-h exposure duration groups were compared to the 12-h exposure duration group ($p \leq 0.01$). Differences were also detected in the 100-mg/L treatment group when the 6-h exposure duration group was compared to the 12-h exposure duration group ($p = 0.01$). No difference was detected in the 100-mg/L treatment group when comparing the 6-h exposure duration group to the 9-h exposure duration group ($p = 0.29$) or when comparing the 9-h exposure duration group to the 12-h exposure duration group ($p = 0.10$).

### Benthic Injection Trials

Mean survival of zebra mussels in the Lake Carlos BI trial SDP-treated groups did not differ ($p = 0.93$) and was 18.1 and 18.0 percent in the 50- and 100-mg/L treatment groups, respectively. Mean survival of zebra mussels in the Shawano Lake BI trial SDP-treated groups differed ($p < 0.01$) and was 2.9 and 0.9 percent in the 50- and 100-mg/L treatment groups, respectively. Survival of zebra mussels in BI SDP-treated groups for both trials differed ($p < 0.01$) from zebra mussel survival in the control groups.

The survival of zebra mussels in the BI trials was compared to the survival of zebra mussels in the respective WWC trial 12-h exposure duration group. Survival of zebra mussels assigned to the Lake Carlos 12-h WWC trial SDP-treated groups differed ($p < 0.02$) from the survival of zebra mussels assigned to the Lake Carlos 12-h BI trial SDP-treated groups; however, after modification of the benthic injection application technique, no difference ($p = 0.22$) was detected between the survival of zebra mussel assigned to the Shawano Lake WWC 12-h exposure group and the Shawano Lake BI trial.

## Conclusions

In this study, the application of the spray dried powder (SDP) formulation of *Pseudomonas fluorescens* (strain CL145A) at 50 and 100 mg/L (based on active ingredient) for 6–12 hours to test tanks containing lake water using either a whole water column (WWC) or benthic injection (BI) application technique significantly reduced the survival of zebra mussels. Mean survival of zebra mussels in the WWC SDP-treated groups did not exceed 12.6 percent for either trial at any exposure duration. No difference in zebra mussel survival was detected between the WWC 50- and 100-mg/L treatment groups in the Lake Carlos trial or in the Shawano Lake trial when compared at the same exposure duration. Mean survival of zebra mussels in the BI SDP-treated groups did not exceed 18.1 percent in the Lake Carlos trial and 2.9 percent in the Shawano Lake trial. After modification of the BI application method for the Shawano Lake trial, survival of zebra mussels in the BI trial did not differ ($p = 0.22$) compared to survival of zebra mussel in the Shawano lake WWC 12-h exposure group. In this study, the amount of SDP applied during the BI trials was 50 percent of that applied during the WWC trials. This study demonstrates that SDP has potential for use in managing dreissenid mussels in limited, open-water environments and that a benthic injection application technique to reduce the amount of SDP required to induce zebra mussel mortality may be successful in quiescent waters.

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# Appendix 1. Study Protocol, Amendments, and Datasheets

**Protocol Title:**

Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates

**Study Number: AEH-12-PSEUDO-04**

**Test Facilities and Study Sponsor**
Upper Midwest Environmental Sciences Center (UMESC)
Mobile Research Laboratory
US Geological Survey
2630 Fanta Reed Rd.
La Crosse, Wisconsin 54603

**Proposed Experimental Start Date:** August 2012
**Proposed Experimental Termination Date:** January 2013

**Protocol Approval**
Reviewed by:

| | | | |
|---|---|---|---|
| [redacted] | 2 Jun 2012 | [redacted] | 8/8/12 |
| Mark P. Gaikowski, M.A. Supervisory Biologist | Date | Michael D. Jawson, Ph.D. Center Director | Date |

Approved by:

| | |
|---|---|
| [redacted] | 8/8/12 |
| [redacted]ma, B.A. Study Director | Date |

File Folder: 3

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TABLE OF CONTENTS

1. INTRODUCTION:

Native freshwater mussel populations of North America were historically considered the most diverse in the world with about 297 recognized taxa consisting of 281 species and 16 subspecies (Williams et al., 1993). Mussels are largely sedentary in nature, relying on movement of host fish during glochidial attachment as means of transport. They are thus particularly vulnerable to a variety of anthropogenic influences including habitat degradation and alteration, pollution and overharvest. A Nature Conservancy survey (Master 1990) found 55% of North America's mussels as extinct or imperiled compared to 7% of terrestrial species, even though terrestrial species traditionally receive far greater attention. Projections in 1999 (Ricciadi and Rasmussen, 1999) suggested that at least 127 imperiled mussel species will be lost in the next 100 years – a conservative extinction rate of 6.4% per decade given it did not take into account extirpations caused by invasive dreissenid mussels (zebra *Dreissena polymorpha* and quaqqa *D. bugensis* mussels).

Concerns for native mussels in the Southeast are potentially even greater given that only 25% of the 269 species historically present are reported as stable compared to the 13% presumed extinct and the 28, 14 and 18% listed, respectively, as endangered, threatened or of special concern. (Neves et al,1997)

Many unionid mussels in North America were imperiled prior to epizoic colonization by zebra and quaqqa mussels though the introduction of dreissenid mussels have dramatically heightened concerns for the continued survival of native mussels. Zebra mussels were reported to be responsible for the extirpation of unionids from waters in Europe as early as 1937 (Sebestyen, 1937). Severe declines in unionid abundance in Europe (Karatayev and Burlakova, 1995; Burlakova, 1998) and North America (Haag et al, 1993; Nalepa, 1994; Ricciardi et al., 1996) have since been well documented in the literature.

The 1973 Endangered Species Act (ESA) brought forth the need to recognize, protect and recover rare mussels in the United States. The United States Fish and Wildlife Service (USFWS) develops recovery plans for threatened and endangered species which utilize a range of tools to promote recovery of the species including restoring and acquiring critical habitat, removing introduced or invasive species and captive propagation and release into historic ranges.

As of 2004, mussel propagation work was being conducted in several different facilities in 7 states as well as in Ontario, Canada (Neves, 2004). The Genoa National Fish Hatchery (GNFH) in Wisconsin has been involved in mussel recovery since 2000, releasing tens of thousands of propagated subadult Higgins eye pearlymussel (*Lampsilis higginsii*) for recovery efforts. The GNFH produces subadult mussels using cage culture techniques. This technique involves placing glochidia laden host fish into submerged cages within natural water bodies such as the Mississippi and St. Croix Rivers. The fish are released from the cages after mussel excystment and the mussels are allowed to grow on the cage bottom for an additional 6-18 months before being harvested. Areas that were previously successful in rearing mussels using this technique have been abandoned due to the colonization and proliferation of zebra mussels.

Biologists at the New York State Museum (NYSM) Field Research Laboratory have been researching dreissenid mussel control techniques since 1991. They discovered that components of a strain of common bacterium isolated from soils (*Pseudomonas fluorescens* [*Pf*-CL145A]) are capable of causing mortality in zebra mussels. Marrone Bio Innovations (MBI; Davis, CA) is currently developing a spray dried formulation of this bacterium called MBI-401SDP. A formulation of *Pf*-CL145A was recently registered with the USEPA for use within closed systems such as power generating plant cooling systems. The NYSM has partnered with the USFWS (Genoa NFH) and United States Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) to determine the suitability of this product for open water zebra mussel control applications including treatment of native mussel propagation cages or native mussel beds.

Naturally occurring surface waters may be unique in their chemical and biological properties which may affect the efficacy of applied control agents such as *Pf*-CL145A. The research to be completed according to this protocol will assess the efficacy of various concentrations and treatment durations of *Pseudomonas fluorescens* (*Pf*-CL145A) for controlling settled zebra mussels (*D. polymorpha*) in open waters.

2. PROTOCOL OBJECTIVE:

To assess the efficacy of various exposure concentrations and treatment durations of *Pseudomonas fluorescens* (*Pf*-CL145A) spray dried powder (SDP) formulation for controlling settled zebra mussels (*D. polymorpha*) in open waters.

3. STUDY SCHEDULE:

3.1 Proposed initiation: August 2012
3.2 Schedule of events: A proposed schedule of events is provided in Table 1.
3.3 Proposed completion date: January 2013

Table 1. Proposed Schedule of Events

| Date | Activity |
|---|---|
| August 2012-Sept 2012 | substrate exposures |
| August 2012-October 2012 | substrate assessment |
| January 2013 | final report submission |

4. STUDY DESIGN:

4.1 General Description:

Zebra mussel-encrusted, perforated aluminum substrates (15.2 cm x 15.2 cm x 2.5 cm) previously placed (10-11/2011and/or 5/2012) in two Minnesota and one Wisconsin water body will be exposed to varying concentrations and treatment durations of *Pf*-CL145A SDP formulation. The exposures will be conducted at Lake Carlos and Lake Pepin in Minnesota and Lake Shawano in Wisconsin with the assistance of MN or WI Department of Natural Resources. Replicated exposures will be conducted adjacent to each water body in an enclosed research trailer. Test water will be drawn from the adjacent water body. *Pf*-CL145A SDP formulation will be added to the water in the treatment tanks. The water containing *Pf*-CL145A will be disposed of in compliance with the regulations of the respective DNR. Untreated water used pre- and post-exposure will be returned to the water body. The exposed substrates will be individually tagged and returned to the water body for approximately 3-4 weeks to assess post-exposure latent mortality.

4.2 Experimental Design:

In October and November of 2011 and again in May 2012 (depending on site) zebra mussel attachment substrates (0.063" thick, type 3003, perforated aluminum sheeting [3/16" hole, 51% open area] 15.2 cm x 15.2 cm trays with 2.5 cm sides) were nested together in groups of 10 trays per stack (separated ~ 2 cm with wooden blocking and zipped tied in place). Just prior to nesting, the trays were seeded by placing approximately 100-200 previously collected zebra mussels on the trays. The zebra mussels were collected (≤ 6-h prior to use) by severing their byssal threads from rocks and other surfaces (e.g., sticks, native mussels, etc) with a scalpel; zebra mussels were maintained in a cooler until placed in an attachment substrate. At each location, approximately 60 nested trays were placed in a wire mesh cage (~ 0.9 m long x 0.6 m wide x 0.45 m high) and placed in ~ 2 m of water for zebra mussel attachment and overwintering. .

Substrate trays will be removed from the cages and placed in a semi-rigid plastic mesh bag (~20.3 x 25.4 x 5.1 cm; ~0.31 x 0.31 cm openings) and sealed with zip ties. Each substrate bag will uniquely tagged and randomly assigned to one of 9 test tanks within the mobile research trailer. At least 3 substrate bags and trays will be placed in each test tank containing up to 325 L of filtered (200 µm) surface water. Flowing, filtered surface water will be supplied to the test tanks at a rate sufficient to achieve ≥1 tank exchange per hour. The substrate bags and trays will be acclimated to conditions within the test tanks for ≥ 12-h prior to administering a single *Pf*-CL145A SDP exposure. Treatment concentration (e.g. 0 [control], 50 or 100 mg/L) will be randomly assigned to each test tank (n=9) and each test tank will serve as an exposure replicate (3 replicates per treatment concentration). For each exposure day, a single treatment duration (ie: 6, 9 or 12-h) will be assigned for all treatment concentrations. At exposure termination the tanks will be drained of treated water, rinsed and refilled with flowing filtered surface water. Within 24 h of exposure termination, the substrate bags and trays will be removed from the test tanks and returned to the wire mesh cages. The cages will then be placed in ~ 2 m of water in the lake or river for a 3-4 week observation period. After 3-4 weeks (dependent on temperature) the cages will be removed from the water body and mussels enumerated for survival. After enumeration all test animals will be euthanized.

Figure 1. Flow chart of major study activities

Substrate trays are removed from the water body, placed in a sealed rigid plastic mesh bag and a unique indentification tag is placed on each bag

Substrate bags containing trays are randomly assigned to 1 of 9 exposure tanks, a minimum of 3 bags/trays are placed in each tank and a constant supply of surface water is supplied to each tank at ≥ 1 tank exchange/h

Figure 1. Continued.

Bags/trays are acclimated to test conditions for ≥ 12-h; water inflow is ceased and static random exposure treatments are administered by the addition of appropriate amounts of *Pf*-CL145A SDP formulation; supplemental aeration is supplied.

Treatments will be a static exposure of 0 (control), 50 or 100 mg/L *Pf*-CL145A SDP formulation active ingredient (A.I.) with either a 6, 9 or 12-h exposure duration. Exposure concentrations will be randomly assigned. For each exposure day, a single exposure duration (6, 9 or 12-h) will be assigned with 3 replicates for each exposure concentration of 0 (control) 50, or 100 mg/L.

At exposure termination, water flow is resumed, tanks are flushed and rinsed. ≤ 24-h post exposure termination the bags/trays are removed from the exposure tanks and the trays are placed in holding cages in ~ 2m of water

The cage positions are marked with GPS and a buoy, if required. After 3-4 weeks, the cages are removed from the lake and each tray is assessed for mortality, after assessment all animals are euthanized.

All equipment is thoroughly decontaminated prior to removal from the test site

## 5. STUDY PROCEDURES

### 5.1 Test Animals

5.1.1 Description:

5.1.1.1 Age – < 18 months

5.1.1.2 Sex – Test animals will be used without regard to sex.

5.1.1.3 Species – (zebra mussel, *Dreissena polymorpha*)

5.1.2 Number of animals: Approximately 3,000-5,000 mussels, consistent with the objective of the study and contemporary scientific standards.

5.1.3 Source of animals: Animals will be collected from test location surface waters.

5.1.4 Inclusion criterion: Only trays with sufficient numbers (n > 30) of attached zebra mussels in apparent good health will be used.

5.1.5 Acclimation: Mussels will be acclimated to conditions for ≥ 12-h prior to exposure initiation.

5.1.6 Distribution to exposure tanks: One bag/tray will be distributed per exposure tank according to a predetermined randomization scheme in separate rounds. A minimum of 3 trays will be placed in each tank using a minimum of 3 distribution rounds.

5.1.7 Feeding: No supplemental feed will be offered throughout the acclimation, exposure and post-exposure holding periods.

5.2 Water Chemistry

5.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded in each exposure tank during the acclimation, exposure and post-exposure holding periods. Dissolved oxygen will be measured and recorded at least once during the pre- and post-exposure periods and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination (UMESC SOP AEH 394 or equivalent).

5.2.2 Temperature: Temperature will be measured and recorded in each exposure tank during the acclimation, exposure and post-exposure holding periods. Temperature will be measured and recorded at least once during the pre- and post-exposure periods and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination.

5.2.3 pH: pH will be measured and recorded in each exposure tank during the acclimation, exposure and post-exposure holding periods. pH will be measured and recorded at least once during the pre- and post-exposure periods and at least twice during the exposure with the last measurement observed ≤ 30 minutes prior to exposure termination (UMESC SOP AEH 335 or equivalent).

5.2.4 Hardness: Hardness will be measured and recorded prior to exposure initiation (UMESC SOP AEH 712).

5.2.5 Alkalinity: Alkalinity will be measured and recorded prior to exposure initiation (UMESC SOP AEH 706).

5.2.6 Conductivity: Conductivity will be measured and recorded prior to exposure initiation (UMESC SOP AEH 188 or equivalent).

5.2.7 Ammonia: Samples for total ammonia-nitrogen will be collected at the termination of the exposure period for each exposure replicate. Ammonia samples will be filtered through a 0.45 micron syringe filter, acidified (~pH 2.5) with sulfuric acid and then stored at ~4°C until analyzed by the UMESC Long Term Resources Monitoring (LTRM) Water Quality Laboratory using the automated phenate method.

5.3 Disposal: All live mussels at the end of the post-exposure observation period will be euthanized and disposed by incineration or according to other state or local requirements.

5.4 Study facilities:

5.4.1 Test Facility

U.S. Geological Survey, Upper Midwest Environmental Sciences Center
Mobile Research Laboratory
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

5.4.1.1 Test location: Lake Carlos, MN; Lake Pepin, MN; and Lake Shawano, WI

5.4.1.2 Exposure system: The test system consists of nine 76.2 cm diameter x 91.4 cm deep fiberglass circular exposure tanks in two rows of 4 or 5 tanks. The tanks are supplied surface water through a pump, filter and headbox system. Each exposure tank will receive a continuous supply of water from a headbox during the pre- and post-exposure periods. Each tank will be uniquely identified to allow for identification treatment type and replicate number. Coding procedures will be documented in the study records.

5.4.1.3 Aeration: Supplemental aeration will be supplied during the acclimation, *Pf*-CL145A SDP exposures and the post exposure observation periods.

5.4.1.4 Water supply: Filtered surface water will be supplied continuously (~5 L/min) to achieve a ~ tank-volume exchange/h during the pre- and post-exposure periods. Water supply will be interrupted during the *Pf*-CL145A SDP exposures.

5.4.1.5 Water discharge: Untreated water will be returned to the surface water supply source. *Pf*-CL145A SDP treated water will be mechanically and/or carbon filtered and/or collected for disposal as required by DNR regulation.

5.4.1.6 Lighting: Overhead lighting (~16 h light:8 h dark) will be provided.

5.5 Observations:

5.5.1 Mortality: Zebra mussels that are gapping and do respond to tactile stimuli by shell closure will be coded as a mortality. Zebra mussels that have closed shells or respond to tactile stimuli by shell closure will be coded as alive.

5.6 Treatment administration:

5.6.1 Treatment: Each treatment will consist of three *Pf*-CL145A SDP concentrations (ie: 0 [control], 50 or 100 mg/L A.I.) with a single exposure duration (6, 9 or 12-h). All treatment concentrations will have three replicate exposure tanks. A minimum of 3 zebra mussel-encrusted substrate trays will be bagged and placed in each test replicate.

5.6.2 Route of administration: Exposures will be initiated by addition of an appropriate amount of a *Pf*-CL145A SDP stock solution. The tank will be gently mixed to achieve a uniform distribution of test material.

5.6.3 Concentration verification: Concentration will be determined spectrophotometrically. A standard curve will be prepared using a known mass of *Pf*-CL145A SDP. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Barnstead/Thermolyne Corporation Model: Turner SP-830 Plus Beckman spectrophotometer (UMESC SOP AEH 302).

## 6. DATA ANALYSIS

6.1 Experimental unit: The experiment unit will be the exposure tank.

6.2 Number of exposures and replicates: There will be a total of 3 treatment levels (0 [control], 50 and 100 mg *Pf*-CL145A/L) and 3 treatment durations (6, 9 and 12-h) for each treatment level. There will be a total of 3 independent tanks for each treatment concentration and duration which will serve as the replicates. Each treatment concentration will be run concurrently for a single treatment duration. The trays from all treatment durations will be assessed for mortality at the same post exposure evaluation period.

6.3 Statistical methodology:

Survival data will be analyzed using a generalized linear mixed model (SAS PROC GLIMMIX). In every analysis, the exposure tank will be treated as the experimental unit. The change in proportion of survivors will be analyzed using a generalized linear mixed model where the distribution is binomial and the link used is the logit function.

If a significant effect of treatment is identified then pairwise comparison tests will be completed to compare each treatment group to the control group using unadjusted least squares means.

6.4 Statistical significance: Statistical significance will be declared at $p < 0.05$.

6.5 Other data analyses: Statistical methods for other study data collected will include calculation of means, standard deviations and coefficients of variation. The statistical procedures used will be described in detail in the final study report.

7. PERSONNEL

7.1 Study Director: James A. Luoma, B.A.

7.1.1 Address: Upper Midwest Environmental Sciences Center, US Geological Survey, 2630 Fanta Reed Rd., La Crosse, Wisconsin 54603

7.1.2 Contact: Tel: (608) 781-6391, Fax: (608) 783-6066; jluoma@usgs.gov

7.1.3 Training and experience: CV on file at UMESC.

7.2 Other personnel involved in study: Technical staff involved in the study will be identified in the study raw data to include study function. UMESC technical staff training and experience will be documented in CVs included in the study raw data.

8. DISPOSITION/STORAGE

8.1 Study Records: All data generated in the study will be recorded in bound laboratory notebooks, electronic files or kept in file folders. All data sheets, file folders, laboratory notebooks and computer disks will be encoded with the study number when the data are generated. Raw data, laboratory notebooks and electronic files (including a CD-ROM containing the annotated SAS program used for the statistical analysis, the data files, SAS log and SAS output files) generated by UMESC and contract laboratory reports will be filed in the UMESC archives (SOP No. GEN 007) of the Upper Midwest Environmental Sciences Center, La Crosse Wisconsin, before the final report is signed by the Study Director. The final report will then be signed and archived.

9. AMENDMENT/DEVIATIONS TO THE PROTOCOL

9.1 Protocol amendments: A signed copy of the Study Protocol will be retained on-site. Proposed amendments to the protocol shall be brought to the attention of UMESC Management. When the Study Director and Management agree verbally, the study can proceed with the change. As soon as possible, the Study Director will then prepare a written protocol amendment that is signed by the Study Director and Branch Chief. The amendment then becomes an official part of the protocol.

9.2 Protocol deviations: All deviations from this approved protocol will be documented and reviewed by the Study Director. The Study Director will make a judgment on the impact of the deviations. The Study Director will notify Management, as soon as possible, of any deviations to the protocol, including their impact on the study.

10. INVESTIGATIONAL TEST ARTICLE

10.1 Test Substance(s): *Pseudomonas fluorescens* (*Pf*-CL145A) SDP formulation

10.1.1 Chemical name: *Pseudomonas fluorescens* (*Pf*-CL145A)

10.1.2 Trade name: Zequanox

10.1.3 Active ingredients: *Pseudomonas fluorescens* (*Pf*-CL145A) is the sole active ingredient, 50% active by weight.

10.1.4 Source: Marrone Bio Innovations (MBI); Davis, CA

10.1.5 Lot number: Multiple lots are expected to be used during the exposures. Lot number(s) will be included in the test chemical log books, lab notebook, and study files.

10.1.6 Expiration date: As determined by the manufacturer. An aliquot of each lot tested will be returned to the NYSM or MBI at the conclusion of exposures for post-exposure zebra mussel bioassay tests (the standard testing protocol to assess *Pseudomonas fluorescens* [*Pf*-CL145A] formulation activity). Results of these confirmation bioassays will be used to validate the retention of activity of the *Pseudomonas fluorescens* (*Pf*-CL145A) SDP and will be included in the study files when available.

10.1.7 Storage during study: test chemical will be stored refrigerated. Test material will be transported in a cooler with ice packs to maintain proper storage temperature (4-10 °C)

10.1.8 A NIOSH approved respirator will be used when preparing stock solutions to avoid inhalation. Protective eyewear, gloves and lab coats will be worn at all times when working with the test substance.

11. ADVERSE EVENTS: Any adverse event will be recorded in the study logbook and the Study Director will be notified.

12. BIOSECURITY PROCEDURES

12.1 General Procedures: All personnel involved in the study will review the UMESC biosecurity (UMESC SOP APP 075) and project HACCP plans. Testing will be conducted in a mobile laboratory with controlled access. All treated effluent water will be mechanically and/or carbon filtered and/or collected for contract disposal according to federal, state or local requirements.

12.2 HACCP Plan: See Appendix 1 for the HACCP plan for this project.

## 13. STANDARD OPERATING PROCEDURES

A complete list of the standard operating procedures used in the study will be included in the study guide. The follow SOP's were cited in this protocol:

UMESC SOP APP 075 – Procedures to Minimize the Risk of Transfer of Pathogens and Invasive Species
UMESC SOP AEH 188 – Accumet Portable Waterproof Conductivity meter Model # AP75
UMESC SOP AEH 302 – Instrument Operating Procedure: Barnstead/Thermolyne Corporation Model: Turner SP-830 Plus Beckman spectrophotometer Serial # 1365070560781
UMESC SOP AEH 335 – Beckman Portable pH/mV Meter, Model 250
UMESC SOP AEH 394 – YSI Handheld Dissolved Oxygen Meter, Model 55/12FT, Serials 94C17261 & 97F0837AG
UMESC SOP AEH 706 – Determination of Total Alkalinity by the Titrimetric (pH 4.5) Method
UMESC SOP AEH 712 – Determination of Total Hardness

## 14. REFERENCES.

Burlakova, L.E., 1998. Ecology of *Dreissena polymorpha* (PALLAS) and its role in the structre and function of aquatic ecosystems. Candidate dissertation, Zoology Institute of the Academy of Science Republice Belarus, 168 p. (in Russian) in Burlakova L.E., A.Y. Karatayev and D. K. Padilla. 2000. The impact of Dreissena polymorpha (PALLAS) invasion on Unionid bivalves. Internat. Rev. hydrobiol. 85 (5-6):529-541.

Haag, W.R., D.L. Berg, D.W. Garton, and J.L. Farris, 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (Dreissena polymorpha) in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 50(1):13-19.

Karatayev, A.Y. and L.E. Burlakova, 1995. Present and future patterns in Dreissena population development in the Narochanskaya lakes system. Vestisi Akad. Navuk Belarusi. Ser. Biyol. Navuk 3: 95098 (in Belarussian) in Burlakova L.E., A.Y. Karatayev and D. K. Padilla. 2000. The impact of Dreissena polymorpha (PALLAS) invasion on Unionid bivalves. Internat. Rev. hydrobiol. 85 (5-6):529-541.

Master, L., 1990. The imperiled status of North American aquatic animals. Biodiversity Network News 3(3):5-8.

Napela, T.F., 1994. Decline of native unionid bivalves in Lake St. Clair after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51:2227-2233.

Neves R. J., 2004. Propagation of endangered freshwater mussels in North America. Journal of Conchology, special publication 3:69-80.

Neves, R.J., A.E. Bogan, J.D. Williams, S.A. Ahlstedt, and P.W. Hartfield, 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. Pages 43-85 in G.W. Benz and D.E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Southeastern Aquatic Research Institute, Lenz Design and communications, Decatur, Georgia.

Ricciardi, A. F.G. Whoriskey, and J.B. Rasamussen, 1996. Impact of the *Dreissena* invasion on native unionid bivalves in the upper St. Lawerance River. Can. J. Fish. Aquat. Sci. 53:1434-1444.

Ricciardi A., and J.B. Rasmussen, 1999. Extinction rates of North American freshwater fauna. Conservation Biology. Vol. 13 (5):1220-1222.

Sebestyn, O., 1937. Colonization of two new fauna-elements of Pontus-origin (*Dreissena polymorpha* Pall. And *Corophuim curvispinum* G.O. Sars forma devium Wundsch) in Lake Balaton, Verh. Int. Ver. Theor. Angew. Limnol. 8:169-182 in Burlakova L.E., A.Y. Karatayev and D. K. Padilla. 2000. The impact of Dreissena polymorpha (PALLAS) invasion on Unionid bivalves. Internat. Rev. hydrobiol. 85 (5-6):529-541.

Williams, J.D., M.L. Warren Jr., K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9):6-22.

## 15. APPENDIX.

### 15.1 Appendix 1. HACCP PLAN for the study Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates

**Step 1 – Activity Description**

| | |
|---|---|
| Facility: US Geological Survey-Upper Midwest Environmental Sciences Center mobile research laboratory | Site: Various – MN and WI |
| Site Coordinator: Jim Luoma<br>Site Manager: Mark Gaikowski | Activity: Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates |
| Address: 2630 Fanta Reed Road<br>La Crosse WI, 54601 | |
| Phone: 608-781-6322 | |

| Project Description |
|---|
| The objective of this study is todetermine the efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates |

**Step 2 – Potential Hazards: Species which may potentially be moved/introduced**

| |
|---|
| **Vertebrates:**<br>Multiple species of freshwater fish, eggs and gametes found in the Mississippi River and Great Lakes Basin. |
| **Invertebrates:**<br>Zebra mussel (*Dreissena polymorpha*)<br>Faucet snail (*Bithynia tentaculata*)<br>Multiple endemic species found in the Mississippi River and Great Lakes Basin |
| **Plants:**<br>Eurasian water milfoil *Myriophyllum spicatum*<br>Multiple endemic species found in the Mississippi River and Great Lakes Basin |
| **Other biologicals (disease, pathogen, parasite):**<br>Largemouth Bass Virus<br>Spring Viremia of Carp Virus<br>Bluegill Virus<br>Infectious Pancreatic Necrosis Virus<br>Viral Hemorrhagic Septicemia<br>Furunculosis *Aeromonas salmonicida*<br>Enteric Redmouth Disease *Yersinia ruckeri*<br>Bacterial Kidney Disease *Renibacterium salmoninarum*<br>Other Assorted parasites/pathogens found in the Mississippi River and Great Lakes Basin |
| **Other:**<br>NA |

**Step 3 – Flow Diagram**

**Flow diagram outlining sequential tasks to complete activity/project**

| | |
|---|---|
| Task 1 | UMESC mobile research laboratory is brought to test site and setup, water flow initiated |
| Task 2 | Zebra mussel substrate trays are obtained from surface waters and moved into mobile laboratory |
| Task 3 | Substrate trays are acclimate to test conditions with flowing filtered surface water |
| Task 4 | Substrate trays are exposed to test article |
| Task 5 | Substrate trays are placed in holding cages and returned to surface waters for post-exposure holding |
| Task 6 | Mobile trailer, test tanks, hoses, pump and other equipment is decontaminated and returned to UMESC |
| Task 7 | Holding cages and substrate trays are removed from surface waters and assessed for zebra mussel survival |
| Task 8 | Zebra mussels are removed from substrate trays and euthanized |
| Task 9 | All remaining equipment, cages and substrate trays are decontaminated and returned to UMESC |

| 1<br>Tasks<br>(from HACCP Step 3 - Flow Diagram) | 2<br>Potential hazards identified in HACCP Step 2 | 3<br>Are any potential hazards probable? (yes/no) | 4<br>Justify evaluation for column 3 | 5<br>What control measures can be applied to prevent undesirable results? | 6<br>Is this task a critical control point? (yes/no) |
|---|---|---|---|---|---|
| Task 1<br><br>UMESC mobile research laboratory is brought to test site and setup, water flow initiated | Vertebrates | yes | Surface water contains multiple vertebrate species | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces (ie: floors, tanks, hoses, pumps, screens, nets, etc) with steam, chemosterilants, or other approved methods prior to removal from previous location. Inspect all equipment prior to set up and repeat decontamination if warranted. | Yes |

| | | | | | |
|---|---|---|---|---|---|
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces (ie: floors, tanks, hoses, pumps, screens, nets, etc) with steam, chemosterilants, or other approved methods prior to removal from previous location. Inspect all equipment prior to set up and repeat decontamination if warranted. | Yes |
| | Plants | yes | Surface water contains multiple plant species including AIS | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces (ie: floors, tanks, hoses, pumps, screens, nets, etc) with steam, chemosterilants, or other approved methods prior to removal from previous location. Inspect all equipment prior to set up and repeat decontamination if warranted. | yes |
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces (ie: floors, tanks, hoses, pumps, screens, nets, etc) with steam, chemosterilants, or other approved methods prior to removal from previous location. Inspect all equipment prior to set up and repeat decontamination if warranted. | yes |

| | | | | | |
|---|---|---|---|---|---|
| Task 2 Zebra mussel substrate trays are obtained from surface waters and moved into mobile laboratory | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates prior to transfer into mobile research laboratory. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Physical removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |

| | | | | | |
|---|---|---|---|---|---|
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |

| | | | | | |
|---|---|---|---|---|---|
| Task 3<br><br>Substrate trays are acclimate to test conditions with flowing filtered surface water | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | no |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Physica removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | no |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | no |

| | | | | | |
|---|---|---|---|---|---|
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |

| | | | | | |
|---|---|---|---|---|---|
| Task 4<br><br>Substrate trays are exposed to test article | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Physical removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |

| | | | | | |
|---|---|---|---|---|---|
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including al internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |
| Task 5<br><br>Substrate trays are placed in holding cages and returned to surface waters for post-exposure holding | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visib'e vertebrates prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all interna and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |
| | Invertebrates | yes | Surface water contains multip e invertebrate species include AIS | Phys'cal removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | No |

| | | | | | |
|---|---|---|---|---|---|
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | no |

| | | | | | |
|---|---|---|---|---|---|
| Task 6<br><br>Mobile trailer, test tanks, hoses, pump and other equipment is decontaminated and returned to UMESC | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Physical removal of all visible invertebrates. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Others | yes | Surface water has potentia to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |

| | | | | | |
|---|---|---|---|---|---|
| Task 7<br><br>Holding cages and substrate trays are removed from surface waters and assessed for zebra mussel survival | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates prior to transfer into mobile research laboratory. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Physical removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory. Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | yes |

| | | | | | |
|---|---|---|---|---|---|
| Task 8<br><br>Zebra mussels are removed from substrate trays and euthanized | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all interna and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Phys'cal removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination ali equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | yes |

| | | | | | |
|---|---|---|---|---|---|
| Task 9<br><br>All remaining equipment, cages and substrate trays are decontaminated and returned to UMESC | Vertebrates | yes | Surface water contains multiple vertebrate species | Physical removal of all visible vertebrates prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Invertebrates | yes | Surface water contains multiple invertebrate species include AIS | Physical removal of all visible invertebrates not required for testing prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Plants | yes | Surface water contains multiple plant species including AIS | Physical removal of all visible plant material prior to transfer into mobile research laboratory.<br>Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | Yes |
| | Others | yes | Surface water has potential to transfer fish diseases | Assure the thorough decontamination all equipment including all internal and external potentially wetted surfaces with steam, chemosterilants, or other approved methods prior to removal from location. | yes |

**HACCP Plan Form**
**(all CCP's or "yes's" from column 6 of HACCP Step 4 – Hazard Analysis Worksheet)**

| | | | Monitoring | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Critical Control Point (CCP)** | **Significant Hazard(s)** | **Limits for each Control Measure** | **What** | **How** | **Frequency** | **Who** | **Evaluation & Corrective Action(s) (If needed)** | **Supporting Documentation (If any)** |
| Tasks 1, 2, 3,6,7,8 & 9 | Transfer of endemic and AIS including vertebrates, invertebrates, plants and pathogens | Transfer of vertebrates, invertebrates, plants and pathogens must not occur. All equipment must be thoroughly inspected and disinfected prior to site removal and inspected and/or re-decontaminated upon setup at new location. | Equipment disinfection | Mechanical cleaning/removal, pressure washing, steam cleaning, chemosterilant or other approved methods. | Prior to equipment arrival, upon deployment, upon movement from/to surface water and prior to departure | Technicians /lead field supervisor | Supervisor and staff are responsible for methodical decontamination using established procedures. Corrective actions required to complete decontamination must be performed prior to any equipment movement from test location. Decontamination and inspection must be completed before equipment removal from test location | Records in log books all procedures used for decontamination |

| | |
|---|---|
| **Facility:** Upper Midwest Environmental Sciences Center Mobile Research Laboratory | **Activity:** Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates |
| **Address:** 2630 Fanta Reed Road, La Crosse, WI 54601 | |
| **Signature:** HACCP Plan was followed. | **Date:** |

File Folder: 3

Item Number: 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

Date: August 13, 2012

To: The Record Study Number AEH-12-PSEUDO-04

Subject: Amendment 1- Amendment to the study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Revision of Study Protocol, Study # AEH-12-PSEUDO-04 is proposed as detailed on pages 2-7 of this amendment. Revised text is indicated **in bold**.

This amendment details the 1) combining all exposure times into a single exposure bath with removal of substrates replicates at 6, 9 and 12-h; 2) the elimination of supplemental aeration during the exposure period 3) the inclusion of a separate bottom layer treatment exposures if sufficient substrate trays remain after the initial whole tank exposures and 4) the delivery of test material, collection of water samples and water chemistry data.

**Reviewed by:**

[redacted] 13 Aug 12
Mark P. Gaikowski, M.A. Date
Supervisory Biologist
Aquatic Ecosystem Health,
UMESC[1]

[redacted] 13 Aug 2012
for Michael Jawson, Ph.D. Date
Center Director, UMESC

**Approved by:**

[redacted], B.A. 8/13/12
[redacted] UMESC Date

File Folder: 3

Item Number: 2

[1] UMESC: U.S. Geological Survey, Upper Midwest Environmental Sciences Center

Current text:

4.2 Experimental Design:

In October and November of 2011 and again in May 2012 (depending on site) zebra mussel attachment substrates (0.063" thick, type 3003, perforated aluminum sheeting [3/16" hole, 51% open area] 15.2 cm x 15.2 cm trays with 2.5 cm sides) were nested together in groups of 10 trays per stack (separated ~ 2 cm with wooden blocking and zipped tied in place). Just prior to nesting, the trays were seeded by placing approximately 100-200 previously collected zebra mussels on the trays. The zebra mussels were collected (≤ 6-h prior to use) by severing their byssal threads from rocks and other surfaces (e.g., sticks, native mussels, etc) with a scalpel; zebra mussels were maintained in a cooler until placed in an attachment substrate. At each location, approximately 60 nested trays were placed in a wire mesh cage (~ 0.9 m long x 0.6 m wide x 0.45 m high) and placed in ~ 2 m of water for zebra mussel attachment and overwintering. .

Substrate trays will be removed from the cages and placed in a semi-rigid plastic mesh bag (~20.3 x 25.4 x 5.1 cm; ~0.31 x 0.31 cm openings) and sealed with zip ties. Each substrate bag will uniquely tagged and randomly assigned to one of 9 test tanks within the mobile research trailer. At least 3 substrate bags and trays will be placed in each test tank containing up to 325 L of filtered (200 μm) surface water. Flowing, filtered surface water will be supplied to the test tanks at a rate sufficient to achieve ≥1 tank exchange per hour. The substrate bags and trays will be acclimated to conditions within the test tanks for ≥ 12-h prior to administering a single *Pf*-CL145A SDP exposure. Treatment concentration (e.g. 0 [control], 50 or 100 mg/L) will be randomly assigned to each test tank (n=9) and each test tank will serve as an exposure replicate (3 replicates per treatment concentration). For each exposure day, a single treatment duration (ie: 6, 9 or 12-h) will be assigned for all treatment concentrations. At exposure termination the tanks will be drained of treated water, rinsed and refilled with flowing filtered surface water. Within 24 h of exposure termination, the substrate bags and trays will be removed from the test tanks and returned to the wire mesh cages. The cages will then be placed in ~ 2 m of water in the lake or river for a 3-4 week observation period. After 3-4 weeks (dependent on temperature) the cages will be removed from the water body and mussels enumerated for survival. After enumeration all test animals will be euthanized.

5.2 Water Chemistry

5.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded in each exposure tank during the acclimation, exposure and post-exposure holding periods. Dissolved oxygen will be measured and recorded at least once during the pre- and post-exposure periods and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination (UMESC SOP AEH 394 or equivalent).

5.2.2 Temperature: Temperature will be measured and recorded in each exposure tank during the acclimation, exposure and post-exposure holding periods. Temperature will be measured and recorded at least once during the pre- and post-exposure periods and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination.

5.2.3 pH: pH will be measured and recorded in each exposure tank during the acclimation, exposure and post-exposure holding periods. pH will be measured and recorded at least once during the pre- and post-exposure periods and at least twice during the exposure with the last measurement observed ≤ 30 minutes prior to exposure termination (UMESC SOP AEH 335 or equivalent).

5.2.4 Hardness: Hardness will be measured and recorded prior to exposure initiation (UMESC SOP AEH 712).

5.2.5 Alkalinity: Alkalinity will be measured and recorded prior to exposure initiation (UMESC SOP AEH 706).

5.2.6 Conductivity: Conductivity will be measured and recorded prior to exposure initiation (UMESC SOP AEH 188 or equivalent).

5.2.7 Ammonia: Samples for total ammonia-nitrogen will be collected at the termination of the exposure period for each exposure replicate. Ammonia samples will be filtered through a 0.45 micron syringe filter, acidified (~pH 2.5) with sulfuric acid and then stored at ~4°C until analyzed by the UMESC Long Term Resources Monitoring (LTRM) Water Quality Laboratory using the automated phenate method.

5.3 Disposal: All live mussels at the end of the post-exposure observation period will be euthanized and disposed by incineration or according to other state or local requirements.

5.4 Study facilities:

5.4.1 Test Facility

U.S. Geological Survey, Upper Midwest Environmental Sciences Center
Mobile Research Laboratory
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

5.4.1.1 Test location: Lake Carlos, MN; Lake Pepin, MN; and Lake Shawano, WI

5.4.1.2 Exposure system: The test system consists of nine 76.2 cm diameter x 91.4 cm deep fiberglass circular exposure tanks in two rows of 4 or 5 tanks. The tanks are supplied surface water through a pump, filter and headbox system. Each exposure tank will receive a continuous supply of water from a headbox during the pre- and post-exposure periods. Each tank will be uniquely identified to allow for identification treatment type and replicate number. Coding procedures will be documented in the study records.

5.4.1.3 Aeration: Supplemental aeration will be supplied during the acclimation, *Pf*-CL145A SDP exposures and the post exposure observation periods.

5.4.1.4 Water supply: Filtered surface water will be supplied continuously (~5 L/min) to achieve a ~ tank-volume exchange/h during the pre- and post-exposure periods. Water supply will be interrupted during the *Pf*-CL145A SDP exposures.

5.4.1.5 Water discharge: Untreated water will be returned to the surface water supply source. *Pf*-CL145A SDP treated water will be mechanically and/or carbon filtered and/or collected for disposal as required by DNR regulation.

5.4.1.6 Lighting: Overhead lighting (~16 h light:8 h dark) will be provided.

5.5 Observations:

5.5.1 Mortality: Zebra mussels that are gapping and do respond to tactile stimuli by shell closure will be coded as a mortality. Zebra mussels that have closed shells or respond to tactile stimuli by shell closure will be coded as alive.

5.6 Treatment administration:

5.6.1 Treatment: Each treatment will consist of three *Pf*-CL145A SDP concentrations (ie: 0 [control], 50 or 100 mg/L A.I.) with a single exposure duration (6, 9 or 12-h). All treatment concentrations will have three replicate exposure tanks. A minimum of 3 zebra mussel-encrusted substrate trays will be bagged and placed in each test replicate.

5.6.2 Route of administration: Exposures will be initiated by addition of an appropriate amount of a *Pf*-CL145A SDP stock solution. The tank will be gently mixed to achieve a uniform distribution of test material.

5.6.3 Concentration verification: Concentration will be determined spectrophotometrically. A standard curve will be prepared using a known mass of *Pf*-CL145A SDP. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Barnstead/Thermolyne Corporation Model: Turner SP-830 Plus Beckman spectrophotometer (UMESC SOP AEH 302).

Revised text (**in bold**):

4.2 Experimental Design:

In October and November of 2011 and again in **May and August 2012 (depending on site and observed mussel condition)** zebra mussel attachment substrates (0.063" thick, type 3003, perforated aluminum sheeting [3/16" hole, 51% open area] 15.2 cm x 15.2 cm trays with 2.5 cm sides) were nested together in groups of 10 trays per stack **(separated ~ 2 cm with wooden blocking or staggered to allow open corners and zip tied in place)**. Just prior to nesting, the trays were seeded by placing approximately 100-200 previously collected zebra mussels on the trays. The zebra mussels were collected (≤ 6-h prior to use) by severing their byssal threads from rocks and other surfaces (e.g., sticks, native mussels, etc) with a scalpel; zebra mussels were maintained in a cooler until placed in an attachment substrate. **At each location, approximately 60 nested trays were placed in a wire mesh cage (~ 0.9 m long x 0.6 m wide x**

**0.45 m high) and placed in ~ 2 m of water for zebra mussel attachment.** Substrate trays will be removed from the cages and placed in a semi-rigid plastic mesh bag (~20.3 x 25.4 x 5.1 cm; ~0.31 x 0.31 cm openings) and sealed with zip ties. Each substrate bag will uniquely tagged and randomly assigned to one of 9 test tanks within the mobile research trailer. At least 3 substrate bags and trays will be placed in each test tank containing **350 L** of filtered (200 µm) surface water. Flowing, filtered surface water will be supplied to the test tanks at a rate sufficient to achieve ≥1 tank exchange per hour. **The substrate bags and trays will be acclimated to conditions within the test tanks for ≥ 12-h prior to administering a single whole tank treatment of *Pf*-CL145A SDP.** Treatment concentration (e.g. 0 [control], 50 or 100 mg/L) will be randomly assigned to each test tank (n=9) and each test tank will serve as an exposure replicate (3 replicates per treatment concentration). **For each exposure, 9 trays will be randomly assigned to each of 3 treatment concentration replicates. At each exposure termination time point (6, 9 and 12-h) three randomly selected trays will be immediately removed from each treatment replicate, rinsed with tempered surface water and returned to the wire mesh cages located in ~ 1 m of water in the lake or river. Within 12 h of exposure termination the cages will then be relocated and placed in ~ 2 m of water in the lake or river for a 3-4 week observation period.**

**If sufficient untreated substrate trays remain, an additional exposure will be completed in an identical fashion except that 1) the number of trays per replicate and exposure duration time point will be adjusted according to the number of available substrate and 2) the exposures will be conducted as a bottom layer injection designed to treat the bottom 50% of the water column within the tank. The injection design, number of trays and exposure durations tested will be recorded in the study files.**

After 3-4 weeks (dependent on temperature) the cages will be removed from the water body and mussels enumerated for survival. After enumeration all test animals will be euthanized.

5.2 Water Chemistry

**5.2.1** **Dissolved oxygen: Dissolved oxygen will be measured and recorded in each exposure tank during the acclimation and exposure periods. Dissolved oxygen will be measured and recorded at least once during the acclimation period and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination (UMESC SOP AEH 394 or equivalent).**

**5.2.2** Temperature: **Temperature will be measured and recorded in each exposure tank during the acclimation and exposure period. Temperature will be measured and recorded at least once during the acclimation period and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination.**

**5.2.3** **pH: pH will be measured and recorded in each exposure tank during the acclimation and exposure period. pH will be measured and**

**recorded at least once during the acclimation period and at least twice during the exposure period with the last measurement observed ≤ 30 minutes prior to exposure termination (UMESC SOP AEH 335 or equivalent).**

**5.2.4 Hardness: Hardness will be measured and recorded prior to exposure initiation in each headbox (UMESC SOP AEH 712).**

**5.2.5 Alkalinity: Alkalinity will be measured and recorded prior to exposure initiation in each headbox (UMESC SOP AEH 706).**

**5.2.6 Conductivity: Conductivity will be measured and recorded prior to exposure initiation in each headbox (UMESC SOP AEH 188 or equivalent).**

5.2.7 Ammonia: Samples for total ammonia-nitrogen will be collected at the termination of the exposure period for each exposure replicate. Ammonia samples will be filtered through a 0.45 micron syringe filter, acidified (~pH 2.5) with sulfuric acid and then stored at ~4°C until analyzed by the UMESC Long Term Resources Monitoring (LTRM) Water Quality Laboratory using the automated phenate method.

5.3 Disposal: All live mussels at the end of the post-exposure observation period will be euthanized and disposed by incineration or according to other state or local requirements.

5.4 Study facilities:

5.4.1 Test Facility

U.S. Geological Survey, Upper Midwest Environmental Sciences Center
Mobile Research Laboratory
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

5.4.1.1 Test location: Lake Carlos, MN; Lake Pepin, MN; and Lake Shawano, WI

5.4.1.2 Exposure system: The test system consists of nine 76.2 cm diameter x 91.4 cm deep fiberglass circular exposure tanks in two rows of 4 or 5 tanks. The tanks are supplied surface water through a pump, filter and headbox system. **Each exposure tank will receive a continuous supply of water from a headbox during the acclimation period.** Each tank will be uniquely identified to allow for identification treatment type and replicate number. Coding procedures will be documented in the study records.

**5.4.1.3 Aeration: Supplemental aeration may be supplied during the acclimation period and will not be supplied during the *Pf*-CL145A SDP exposure period. Use of supplemental aeration will be documented in the study record.**

**5.4.1.4 Water supply: Filtered surface water will be supplied continuously (~5 L/min) to achieve a ~ tank-volume exchange/h during the acclimation period. Water supply will be interrupted during the *Pf*-CL145A SDP exposures.**

5.4.1.5 Water discharge: Untreated water will be returned to the surface water supply source. *Pf*-CL145A SDP treated water will be mechanically and/or carbon filtered and/or collected for disposal as required by DNR regulation.

5.4.1.6 Lighting: Overhead lighting (~16 h light:8 h dark) will be provided.

5.5 Observations:

5.5.1 Mortality: Zebra mussels that are gapping and do respond to tactile stimuli by shell closure will be coded as a mortality. Zebra mussels that have closed shells or respond to tactile stimuli by shell closure will be coded as alive.

5.6 Treatment administration:

**5.6.1 Treatment: Each treatment will consist of three *Pf*-CL145A SDP concentrations (ie: 0 [control], 50 or 100 mg/L A.I.) with multiple exposure durations (6, 9 and 12-h). All treatment concentrations will have three replicate exposure tanks. A minimum of 3 zebra mussel-encrusted substrate trays will be bagged and placed in each test replicate. Randomly selected trays will be removed from each test replicate at the end of each exposure duration, rinsed with tempered surface water and returned to the wire mesh holding cages.**

**5.6.2 Route of administration: Exposures will be initiated by addition of an appropriate amount of a *Pf*-CL145A SDP stock solution. In whole tank treatments, the tank will be gently mixed to achieve a uniform distribution of test material. In the bottom injection treatments the appropriate amount of a *Pf*-CL145A SDP stock solution will be delivered with a peristaltic pump at ~1/4 of the water column height (~19 cm) using four suspended delivery tubes.**

**5.6.3 Concentration verification: Concentration will be determined spectrophotometrically. A standard curve will be prepared using a known mass of *Pf*-CL145A SDP. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Barnstead/Thermolyne Corporation Model: Turner SP-830 Plus Beckman spectrophotometer (UMESC SOP AEH 302) or equivalent. Samples collected during the whole tank treatments exposures will be collected from the surface of the exposure replicates. Samples collected during the bottom layer injection exposures will be collected with a peristaltic pump from the bottom at ~1/4 of the water column height (~19 cm) using four suspended collection tubes. The collected water from each delivery tube will be pooled for each replicate tank and analyzed for *Pf*-CL145A SDP concentration. Concentrations will be verified in each replicate within 30 minutes of initial dosing and at 3, 6, 9 and 12-h post-dosing.**

Study Number: AEH-12-PSEUDO-04 Reviewed by: ____________ Date: ____________

File Folder: __________ Lab book/pgs: ________ Verified by: ____________ Date: ____________

## Zebra Mussel Survival

Test Organism: Zebra Mussels Applications Type: ________________ Exposure Date: __________

Test Chemical: Pf-CL145A SDP Lot #: 401P12163C and 401P12164C Mix Days Post-Exp: __________

Test Location: ______________________ Holding cage GPS Coordinates: ________________

| Sample ID | Exposure Time (h) | Concentration (mg/L) | Number Alive | Number Dead | Comments | Date/Initials |
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File Folder: 19 Item Number: 1 Page 1 of 1

Study Number: AEH-12-PSEUDO-04 Reviewed by: ____________ Date: ____________

File Folder: __________ Lab book/pgs: ________ Verified by: ______________ Date: ____________

# Zebra Mussel Lengths

Test Organism: Zebra Mussels Applications Type: __________________ Exposure Date: __________

Test Chemical: Pf-CL145A SDP Lot #: 401P12163C and 401P12164C Mix

Test Location: ______________________________

| Sample ID | Mussel Number | Exposure Time (h) | Conc. (mg/L) | Shell Length (mm) | Comments | Date | Initials |
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File Folder: 19 Item Number: 2 Page 1 of 1

Study Number: AEH-12-PSEUDO-04 Reviewed by: ____________ Date: __________

File Folder: ________ Lab book/pgs: ______________ Verified by: ______________ Date: __________

# Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluroescens* strain 145A

Test Chemical Lot #: 401P12163C and 401P12164C Mix Date Rec'd 7-Aug-12 Exp. Date 21-Jun-12

Test Organism : zebra mussels Test Location: ________________

Instruments Used: ______________________

**Weights of Chemical Samples:**

| Sample ID | Sample Weight | Comments | Date | Initials |
|---|---|---|---|---|
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NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

**Stock Solution Preparation:**

| Sample ID | Dilution Volume (mL) | Dilution Time | Use | Exposure Time | Date | Initials |
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File Folder: 19 Item Number: 3 Page 1 of 1

Study Number AEH-12-PSEUDO-04 File Folder: ____________ Reviewed by: ____________ Date: ______

Lab book/pgs: ____________ Verified by: ____________ Date: ______

# Conductivity and Hardness - Exposure Initiation

Test Organism: Zebra mussels Test Location: ____________ Instruments: ____________

Test Chemical: Pf-CL145A SDP Lot Number: 401P12163C and 401P12164C Mix Exposure Date: ____________

Water Source: ____________ Water filtered: Y / N Filter size: ______ µm

| Head Box ID | Replicate | Conductivity (µS/cm) | mL of 0.01 M EDTA | Multiplication Factor | Hardness[1] (mg/L $CaCO_3$) | Commments | Date | Initials |
|---|---|---|---|---|---|---|---|---|
| | 1 | | | 20 | | | | |
| | 2 | | | 20 | | | | |
| | 3 | | | 20 | | | | |
| | 1 | | | 20 | | | | |
| | 2 | | | 20 | | | | |
| | 3 | | | 20 | | | | |

[1] Hardness in mg/L $CaCO_3$ = (mL of 0.01 M $Na_2EDTA$ titrant added to the sample) x (multiplication factor of 20).

Hardness Sample volume = 50 mL.

File Folder: 19

Item Number: 4

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Study Number AFH-12-PSEUDO-04 File Folder: ______ Reviewed by: ______ Date: ______
Lab book/pgs: ______ Verified by: ______ Date: ______

# Alkalinity - Exposure Initiation

Test Organism: Zebra mussels Test Location: ______ Instruments: ______
Test Chemical: Pf-CL145A SDP Lot Number: 401P12163C and 401P12164C Mix Exposure Date: ______
Water Source: ______ Water filtered: Y / N Filter size: ______ µm

| Head Box ID | Replicate | Initial pH | Initial Temp (°C) | mL of 0.02 N $H_2SO_4$ | Multiplication Factor | Alkalinity[1] (mg/L $CaCO_3$) | Commments | Date | Initials |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 10 | | | | |
| | 2 | | | | 10 | | | | |
| | 3 | | | | 10 | | | | |
| | 1 | | | | 10 | | | | |
| | 2 | | | | 10 | | | | |
| | 3 | | | | 10 | | | | |

Sample volume = 100 mL
(1) Alkalinity in mg/L CaCO3 = (mL 0.02N H2SO4 used) x (Multiplication Factor of 10)

File Folder: 19
Item Number: 5
Page 1 of 1

Study Number: AEH-12-PSEUDO 04
File Folder: ________ Lab book/pgs: ________

Reviewed by: ________ Date: ________
Verified by: ________ Date: ________

# Water Quality - Temperature (°C) Measurements

Test Organism: Zebra Mussels Test Location: ________ Application Type: ________
Test Chemical: Pf-CL145A SDP Lot Number: 401P12163C and 401P12164C Exposure Start Time/Date: ________
Tank Volume (L): ________ Water Type/Source: ________ Water Filtered: Y / N Filter size: ____ µm
Instruments Used: ________ Location of Reading: ________

| Exposure Tank ID | Concentration (mg/L) | Pre-Exposure (1) | Pre-Exposure (2) | Exposure Initial | Exposure 3 hour | Exposure 6 hour | Exposure 9 hour | Exposure 12 hour |
|---|---|---|---|---|---|---|---|---|
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| | Time of reading | | | | | | | |
| | Date and Initials | | | | | | | |

File Folder: 19 Item Number: 6 Page 1 of 1

Study Number: AEH-12-PSEUDO-04
File Folder: ______ Lab book/pgs: ______

Reviewed by: ______ Date: ______
Verified by: ______ Date: ______

# Water Quality - pH Measurements

Test Organism: Zebra Mussels Test Location: ______ Application Type: ______
Test Chemical: Pf-CL145A SDP Lot Number: 401P12163C and 401P12164C Exposure Start Time/Date: ______
Tank Volume (L): ______ Water Type/Source: ______ Water Filtered: Y / N Filter size: ____µm
Instruments Used: ______ Location of Reading: ______

| Exposure Tank ID | Concentration (mg/L) | Pre-Exposure (1) | Pre-Exposure (2) | Exposure Initial | Exposure 3 hour | Exposure 6 hour | Exposure 9 hour | Exposure 12 hour |
|---|---|---|---|---|---|---|---|---|
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| | Time of reading | | | | | | | |
| | Date and Initials | | | | | | | |

File Folder: 19 Item Number: 7 Page 1 of 1

Study Number: AEH-12-PSEUDO-04
File Folder: __________ Lab book/pgs: __________

Reviewed by: __________ Date: __________
Verified by: __________ Date: __________

# Water Quality - Dissolved Oxygen (mg/L) Measurements

Test Organism: Zebra Mussels Test Location: __________ Application Type: __________
Test Chemical: Pf-CL145A SDP Lot Number: 401P12163C and 401P12164C Exposure Start Time/Date: __________
Tank Volume (L): __________ Water Type/Source: __________ Water Filtered: Y / N Filter size: _____µm
Instruments Used: __________ Location of Reading: __________

| Exposure Tank ID | Concentration (mg/L) | Pre-Exposure (1) | Pre-Exposure (2) | Exposure Initial | Exposure 3 hour | Exposure 6 hour | Exposure 9 hour | Exposure 12 hour |
|---|---|---|---|---|---|---|---|---|
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| | Time of reading | | | | | | | |
| | Date and Initials | | | | | | | |

File Folder: 19 Item Number: 8 Page 1 of 1

Study Number: AEH-12-PSEUDO-04
File Folder: ______ Lab book/pgs: ______

Reviewed by: ______ Date: ______
Verified by: ______ Date: ______

# Ammonia Sample Collection - Exposure Termination

Test Organism: Zebra mussels  Test Location: ______ Exposure Date: ______
Test Chemical: Pf-CL145A SDP  Lot Number: 401P12163C and 401P12164C Mix
Instruments Used: ______ Application Type: ______ Location of Sample: ______

| Exposure Tank ID | Concentration (mg/L) | Sampling Time ____ h | | | Comments |
|---|---|---|---|---|---|
| | | pH | Temp (°C) | Date / Initials | |
| | | | | | |
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Note: Approximately 5 mL samples will be collected at 24-hour from each exposure chamber. The samples will be filtered through a 0.45 µm syringe filter. 3 mL of the filtered sample will be acidified with 60 µL of 10% sulfuric acid, and stored at 4°C until analysis. Temperature and pH will be measured when the ammonia samples are collected.

File Folder: 19 Item Number: 9 Page 1 of 1

# Appendix 2. Deviations from the Study Protocol

| Item Number | Item Description | Number of Pages | Report Page Number |
|---|---|---|---|
| 1 | Deviation #1 – Randomization of tank treatment assignment error for Shawano Lake whole water body trial | 1 | 64 |
| 2 | Deviation #2 – Randomization of substrate removal from tanks error | 1 | 65 |
| 3 | Deviation #3 – Total ammonia-nitrogen water samples not collected at the 6 and 9 hour termination during the whole water treatment at Lake Shawano | 1 | 66 |
| 4 | Deviation #4 – Control exposure tanks not analyzed for concentration verification at Lake Carlos | 1 | 67 |
| 5 | Deviation #5 – No curriculum vitae or signature on the verification page for an incidental data collector | 1 | 68 |
| 6 | Deviation #6 – Removal of Lake Pepin test location | 1 | 69 |

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

MEMORANDUM

Date: November 19, 2013
To: The Record Study Number AEH-12-PSEUDO-04

Subject: Deviation 1 to study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Deviation #1 – Randomization of Tank Treatment Assignment Error for Shawano Lake whole water body trial

For unknown reasons the randomization prepared for the experimental tank treatment assignment during the Shawano Lake whole water body trial was not followed. The treatments were applied repetitively in chronological order (e.g. tanks 1, 2, and 3 received 0, 50 and 100 mg/L respectively, tanks 4, 5, and 6 received 0, 50 and 100 mg/L respectively, and tanks 7, 8, and 9 received 0, 50 and 100 mg/L respectively). The tank treatment assignment applied to each tank was verified with stock preparation data (File Folder 07), the water chemistry data (File Folder 11b), and the spectrophotometry data (File Folder 11c).

No adverse impacts are anticipated as a result of this deviation as the test animals (ie: bagged mussel trays) were randomly assigned to each treatment tank. Additionally, each treatment level was conducted in triplicate. Any impacts to the study as a result of this deviation will be addressed in the final report.

[redacted] 19 Nov 13
Date

Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 11/19/13
Date

James A. Luoma, B.A.
Study Director, UMESC

cc: UMESC QAU

Item Number: 3

File Folder: 3

Page 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

MEMORANDUM

Date: November 19, 2013
To: The Record Study Number AEH-12-PSEUDO-04

Subject: Deviation 2 to study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Deviation #2 – Randomization of Substrate Removal from Tanks Error

Due to a programming and proc print error, the randomizations generated for the removal of substrates from exposure tanks 5, 7 and 9 for each trial (i.e., whole water body and bottom injection application for each testing location) were the same as the randomizations prepared for exposure tank 2 (i.e., the data for tank 2 was printed in error for tanks 5, 7 and 9) from each individual trial. Therefore at 6, 9 and 12 h post-dosing initiation, treated substrates were removed from the same location in exposure tanks 2, 5, 7 and 9.

No adverse impacts are anticipated as a result of this deviation as the test animals (ie: bagged mussel trays) were randomly assigned to each treatment tank. Additionally, each treatment level was conducted in triplicate and was randomly assigned to the exposure tanks. Any impacts to the study as a result of this deviation will be addressed in the final report.

[redacted] 19Nov13
Date

Kerry L. Weber, M.S.
Principal Investigator, UMESC

A[redacted] 11/19/13
Date

J[redacted], B.A.
Study Director, UMESC

cc: UMESC QAU

**Item Number:** 4

**File Folder:** 3

Page 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

MEMORANDUM

Date: November 20, 2013
To: The Record Study Number AEH-12-PSEUDO-04

Subject: Deviation 3 to study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Deviation #3 – Total ammonia-nitrogen water samples not collected at the 6 and 9 hour termination during the whole water treatment at Lake Shawano

Section 5.2.7 of study number AEH-12-PSEUDO-04 amended protocol entitled "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates" states that "samples for total ammonia-nitrogen will be collected at the termination of the exposure period for each exposure replicate."

Previous studies (AEH-11-PSEUDO-01, AEH-11-PSEUDO-02 and AEH-12-PSEUDO-03) demonstrated that ammonia levels did not appreciably accumulate during 24 hour static treatments of the commercially produced *Pseudomonas fluorescens* (*Pf*-CL145A). Additionally, ammonia accumulation increases with degradation and, therefore, the greatest concentration would be at the 12 hour termination. Therefore, total ammonia-nitrogen water samples were collected only at the 12 hour exposure termination and not at 6 and 9 hour exposure termination times for the whole water treatment at Lake Shawano.

No adverse impacts are anticipated as a result of this deviation as ammonia levels from the static treatments would be highest at 12 hours. The highest observed un-ionized ammonia concentration at the 12 hour termination of the Lake Shawano whole tank treatment was 0.045 mg/L (Tank 2; 50 mg/L treatment group), a level that should not cause acute ammonia toxicity. Any impacts to the study as a result of this deviation will be addressed in the final report.

[redacted] 20NOV13
Date

Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 11/20/13
Date

[redacted] B.A.
Study Director, UMESC

cc: UMESC QAU

**Item Number:** 5

**File Folder:** 3

Page 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

MEMORANDUM

Date: November 20, 2013
To: The Record Study Number AEH-12-PSEUDO-04

Subject: Deviation 4 to study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Deviation #4 – Control exposure tanks not analyzed for concentration verification at Lake Carlos

Section 5.6.3 of study number AEH-12-PSEUDO-04 amended protocol entitled "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates" states that "concentrations will be verified in each replicate within 30 minutes of initial dosing and at 3, 6, 9 and 12-h post-dosing."

Water samples from the control exposure tanks were not analyzed for *Pf*-CL145A concentration during the Lake Carlos whole water treatment (Tanks 2, 3, and 5) or the Lake Carlos bottom injection treatment (Tanks 3, 6 and 7). Samples were analyzed from all treated exposure replicates at 1, 3, 6, 9 and 12 hours for both whole water and bottom injections treatments.

No adverse impacts are anticipated as a result of this deviation as a linear, zero intercept standard curve was created from dilutions prepared from a 2,000 mg/L *Pf*-CL145A stock solution in 200 µm filtered Lake Carlos water and the spectrophotometer was blanked using 200 µm filtered Lake Carlos water. Additionally, all treatment replicates were isolated, static treatments which precluded any cross contamination. Any impacts to the study as a result of this deviation will be addressed in the final report.

[redacted] 20 Nov 13
Date
Principal Investigator, UMESC

A[redacted] 11/20/13
Date
[redacted], B.A.
Study Director, UMESC

**File Folder:** 3

cc: UMESC QAU

**Item Number:** 6

Page 1 of 1

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

MEMORANDUM

Date: February 10, 2014
To: The Record Study Number AEH-12-PSEUDO-04

Subject: Deviation 5 to study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Deviation #5 – No curriculum vitae or signature on the verification page for an incidental data collector

Data entries were recorded on "Zebra Mussel Survival" forms (File Folder 14d) by an individual with the initials "ATM". The individual was Anna T. Morales, a WI DNR scientist working in Shawano County, Wisconsin. Ms. Morales only participated in the enumeration of zebra mussels during the Shawano assessment conducted on October 10, 2012. Ms. Morales worked under the direct supervision of UMESC Biologists, only enumerated three samples, and completed no other study activities. No curriculum vitae or signature on the verification page were obtained; attempts to locate Ms. Morales were unsuccessful.

No adverse impacts are anticipated as a result of this deviation and any impacts to the study as a result of this deviation will be addressed in the final report.

[redacted] 10 FEB 2014
Written by Date
Todd J. Severson, B.S.
Biologist, UMESC

[redacted] 2/10/2014
Date
[redacted]oma, B.A.
Study Director, UMESC

**File Folder:** 3

**Item Number:** 7

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
**Upper Midwest Environmental Sciences Center**
**2630 Fanta Reed Road**
**La Crosse, Wisconsin 54603**

MEMORANDUM

Date: May 30, 2014

To: The Record Study Number AEH-12-PSEUDO-04

Subject: Deviation 6 to study AEH-12-PSEUDO-04 "Efficacy of *Pseudomonas fluorescens* (*Pf*-CL145A) SDP for controlling settled zebra mussels on artificial substrates"

Deviation #6 – Removal of Lake Pepin test location

A wide spread die-off of *Dreissena polymorpha* (zebra mussels) in the Upper Mississippi River system resulted in the need to cancel test exposures at Lake Pepin in Minnesota. Zebra mussel test animals were unavailable, with extremely few animals being found within approximately 5 river miles of the proposed test site. Exposures were successfully completed at two locations (Lake Carlos, Minnesota and Shawano Lake, Wisconsin), which resulted in sufficient data collection.

There were no adverse impacts as a result of this deviation as sufficient data was collected during the two exposures conducted to provide robust and scientifically defensible conclusions.

[redacted] 30MAY2014
Date
Kerry L. Weber, M.S.
Principal Investigator, UMESC

[redacted] 5/30/2014
Date
James A. Luoma, B.A.
Study Director, UMESC

**File Folder:** 3

**Item Number:** 8

# Appendix 3. Randomization Assignments

| Item Number | Item Description | Number of Pages | Report Page Number |
|---|---|---|---|
| 1 | SAS generated random assignment of treatment to experimental tank (Lake Carlos; whole tank treatment) | 4 | 71 |
| 2 | SAS generated random assignment of trays to test tank/position (Lake Carlos; whole tank treatment) | 8 | 75 |
| 3 | SAS generated random assignment of substrate removal from tanks (Lake Carlos; whole tank treatment | 30 | 83 |
| 4 | SAS generated random assignment of treatment to experimental tank (Lake Carlos; bottom injection treatment) | 4 | 113 |
| 5 | SAS generated random assignment of trays to test tank/position (Lake Carlos; bottom injection treatment) | 8 | 117 |
| 6 | SAS generated random assignment of substrate removal from tanks (Lake Carlos; bottom injection treatment | 30 | 125 |
| 7 | SAS generated random assignment of treatment to experimental tank (Lake Shawano; whole tank treatment) | 4 | 155 |
| 8 | SAS generated random assignment of trays to test tank/position (Lake Shawano; whole tank treatment) | 8 | 159 |
| 9 | SAS generated random assignment of substrate removal from tanks (Lake Shawano; whole tank treatment | 30 | 167 |
| 10 | SAS generated random assignment of treatment to experimental tank (Lake Shawano; bottom injection treatment) | 4 | 197 |
| 11 | SAS generated random assignment of trays to test tank/position (Lake Shawano; bottom injection treatment) | 8 | 201 |
| 12 | SAS generated random assignment of substrate removal from tanks (Lake Shawano; bottom injection treatment | 30 | 209 |

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrat 1
AEH-12-PSEUDO-04
Random assignment of treatment to experimental tanks 8/11/12
Treatment Location/type: Lake Carlos - whole water body JL

AEH-12-PSEUDO-04

| Obs | block | tank | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.04216 | Tank 3 | control |
| 2 | 1 | 4 | 0.04661 | Tank 4 | 50 |
| 3 | 1 | 7 | 0.24380 | Tank 7 | 100 |
| 4 | 1 | 5 | 0.34596 | Tank 5 | control |
| 5 | 1 | 1 | 0.45411 | Tank 1 | 50 |
| 6 | 1 | 6 | 0.55661 | Tank 6 | 100 |
| 7 | 1 | 2 | 0.68773 | Tank 2 | control |
| 8 | 1 | 8 | 0.83316 | Tank 8 | 50 |
| 9 | 1 | 9 | 0.89124 | Tank 9 | 100 |

File Folder: 9a Item Number: 1 Page 1 of 4

Analysis performed by J. Luoma SAS version 9.2 08:59 11AUG12

```
/***********************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 11 August 2012 - JAL  JL
*  Verified by:  ______ (Date:_______)                  page ____ of ____
*  Random allocation of treatment to tank.sas
***********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Lake Carlos - whole tank exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;
proc sort data=fish2;
 by block x;
run;

data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;
proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Lake Carlos - whole water body';
run;
```

AEH-12-PSEUDO-04

Page 2 of 4

```
*  date created  : 11 August 2012 - JAL  8/11/12
*  Verified by:  _______ (Date:_______)   JL          page ____ of ____
*  Random allocation of treatment to tank.sas
**********************************************************************/
```

AEH-12-PSEUDO-04

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Lake Carlos - whole tank exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.FISH has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;

NOTE: There were 9 observations read from the data set WORK.FISH.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=fish2;
 by block x;
run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

Page 3 of 4

AEH-12-PSEUDO-04

```
data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.ASSIGN_TRT_FISH has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Lake Carlos - whole water body';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_FISH.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

8/16/12
[illegible]

FF # 9a
Item No. 1
Pg 4 of 4

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical s 1
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Lake Carlos/whole water tank treatment

8/11/12
JN

AEH-12-PSEUDO-04

Start
1500 h

Finished
1630h

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 3 | 2 | 0.01581 | B | 2B3 |
| 2 | 1 | 1 | 1 | 5 | 0.01848 | A | 5A1 |
| 3 | 1 | 3 | 3 | 3 | 0.01856 | C | 3C3 |
| 4 | 1 | 3 | 2 | 9 | 0.02608 | C | 9C2 |
| 5 | 1 | 2 | 2 | 7 | 0.02978 | B | 7B2 |
| 6 | 1 | 3 | 1 | 7 | 0.03709 | C | 7C1 |
| 7 | 1 | 1 | 3 | 2 | 0.06534 | A | 2A3 |
| 8 | 1 | 2 | 3 | 8 | 0.08637 | B | 8B3 |
| 9 | 1 | 1 | 2 | 9 | 0.09121 | A | 9A2 |
| 10 | 1 | 1 | 2 | 1 | 0.13514 | A | 1A2 |
| 11 | 1 | 1 | 2 | 7 | 0.14899 | A | 7A2 |
| 12 | 1 | 1 | 2 | 2 | 0.14907 | A | 2A2 |
| 13 | 1 | 3 | 1 | 6 | 0.22154 | C | 6C1 |
| 14 | 1 | 2 | 3 | 6 | 0.22497 | B | 6B3 |
| 15 | 1 | 2 | 1 | 7 | 0.23740 | B | 7B1 |
| 16 | 1 | 1 | 3 | 5 | 0.24309 | A | 5A3 |
| 17 | 1 | 3 | 3 | 8 | 0.24872 | C | 8C3 |
| 18 | 1 | 2 | 3 | 1 | 0.24915 | B | 1B3 |
| 19 | 1 | 1 | 2 | 8 | 0.25031 | A | 8A2 |
| 20 | 1 | 1 | 2 | 5 | 0.27193 | A | 5A2 |
| 21 | 1 | 1 | 3 | 7 | 0.27954 | A | 7A3 |
| 22 | 1 | 1 | 3 | 1 | 0.31226 | A | 1A3 |
| 23 | 1 | 3 | 3 | 9 | 0.31388 | C | 9C3 |
| 24 | 1 | 1 | 3 | 4 | 0.32192 | A | 4A3 |
| 25 | 1 | 1 | 1 | 7 | 0.32805 | A | 7A1 |
| 26 | 1 | 3 | 1 | 2 | 0.33235 | C | 2C1 |
| 27 | 1 | 3 | 2 | 4 | 0.34771 | C | 4C2 |
| 28 | 1 | 2 | 1 | 1 | 0.35529 | B | 1B1 |
| 29 | 1 | 3 | 1 | 9 | 0.35723 | C | 9C1 |
| 30 | 1 | 1 | 1 | 4 | 0.36690 | A | 4A1 |
| 31 | 1 | 1 | 1 | 3 | 0.37178 | A | 3A1 |
| 32 | 1 | 1 | 3 | 9 | 0.38632 | A | 9A3 |
| 33 | 1 | 1 | 1 | 2 | 0.39205 | A | 2A1 |
| 34 | 1 | 3 | 1 | 1 | 0.40328 | C | 1C1 |
| 35 | 1 | 1 | 1 | 9 | 0.41738 | A | 9A1 |
| 36 | 1 | 3 | 3 | 6 | 0.41888 | C | 6C3 |
| 37 | 1 | 2 | 1 | 3 | 0.42874 | B | 3B1 |
| 38 | 1 | 1 | 2 | 6 | 0.45875 | A | 6A2 |
| 39 | 1 | 3 | 1 | 3 | 0.45937 | C | 3C1 |
| 40 | 1 | 3 | 3 | 4 | 0.48106 | C | 4C3 |
| 41 | 1 | 2 | 3 | 3 | 0.50277 | B | 3B3 |
| 42 | 1 | 2 | 2 | 8 | 0.51279 | B | 8B2 |
| 43 | 1 | 3 | 1 | 4 | 0.52493 | C | 4C1 |
| 44 | 1 | 3 | 3 | 5 | 0.53590 | C | 5C3 |
| 45 | 1 | 2 | 2 | 3 | 0.55895 | B | 3B2 |
| 46 | 1 | 2 | 3 | 7 | 0.57234 | B | 7B3 |

Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12

File Folder: 9a Item Number: 2

Page 1 of 8

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical s 2
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Lake Carlos/whole water tank treatment 8/1/12 JN

AEH-12-PSEUDO-04

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 47 | 1 | 3 | 2 | 7 | 0.59035 | C | 7C2 |
| 48 | 1 | 1 | 3 | 8 | 0.60064 | A | 8A3 |
| 49 | 1 | 2 | 2 | 4 | 0.60112 | B | 4B2 |
| 50 | 1 | 2 | 2 | 6 | 0.64896 | B | 6B2 |
| 51 | 1 | 1 | 1 | 1 | 0.65358 | A | 1A1 |
| 52 | 1 | 1 | 1 | 8 | 0.65971 | A | 8A1 |
| 53 | 1 | 3 | 2 | 3 | 0.68120 | C | 3C2 |
| 54 | 1 | 1 | 2 | 3 | 0.68828 | A | 3A2 |
| 55 | 1 | 3 | 1 | 8 | 0.68878 | C | 8C1 |
| 56 | 1 | 2 | 2 | 2 | 0.69821 | B | 2B2 |
| 57 | 1 | 2 | 3 | 9 | 0.70845 | B | 9B3 |
| 58 | 1 | 3 | 2 | 8 | 0.71981 | C | 8C2 |
| 59 | 1 | 3 | 2 | 2 | 0.72917 | C | 2C2 |
| 60 | 1 | 2 | 2 | 1 | 0.73674 | B | 1B2 |
| 61 | 1 | 3 | 1 | 5 | 0.74149 | C | 5C1 |
| 62 | 1 | 2 | 2 | 9 | 0.74507 | B | 9B2 |
| 63 | 1 | 3 | 2 | 1 | 0.75888 | C | 1C2 |
| 64 | 1 | 3 | 3 | 1 | 0.77048 | C | 1C3 |
| 65 | 1 | 2 | 3 | 4 | 0.77569 | B | 4B3 |
| 66 | 1 | 2 | 1 | 5 | 0.78253 | B | 5B1 |
| 67 | 1 | 3 | 3 | 2 | 0.78719 | C | 2C3 |
| 68 | 1 | 2 | 3 | 5 | 0.81481 | B | 5B3 |
| 69 | 1 | 2 | 1 | 8 | 0.84781 | B | 8B1 |
| 70 | 1 | 1 | 3 | 6 | 0.85703 | A | 6A3 |
| 71 | 1 | 2 | 2 | 5 | 0.87137 | B | 5B2 |
| 72 | 1 | 2 | 1 | 6 | 0.88824 | B | 6B1 |
| 73 | 1 | 1 | 3 | 3 | 0.89142 | A | 3A3 |
| 74 | 1 | 1 | 1 | 6 | 0.89950 | A | 6A1 |
| 75 | 1 | 3 | 2 | 6 | 0.91486 | C | 6C2 |
| 76 | 1 | 3 | 2 | 5 | 0.93631 | C | 5C2 |
| 77 | 1 | 2 | 1 | 4 | 0.94092 | B | 4B1 |
| 78 | 1 | 3 | 3 | 7 | 0.97448 | C | 7C3 |
| 79 | 1 | 1 | 2 | 4 | 0.97694 | A | 4A2 |
| 80 | 1 | 2 | 1 | 9 | 0.98206 | B | 9B1 |
| 81 | 1 | 2 | 1 | 2 | 0.98594 | B | 2B1 |

Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12

Page 2 of 8

Page ____ of ____ Item Number: ____ File Folder: ____

```
/*****************************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : AUGUST 11, 2012 - JAL
*  Verified by:  _______ (Date:_______)                    page ____ of ____
*  Random allocation of trays to tank.sas
*****************************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2, or 3, e
   round = distribution round, place one tray in the assigned position (9 per test replicate - 3 for

/**********************************************************************************************

/*Location and exposure type: Lake Carlos - Whole tank treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
 if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
  if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
```

```
if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
 if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
  if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
   if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
    if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
     if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
      if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
 if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
  if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
   if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
    if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
     if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
      if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
       if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
        if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
 if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
  if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
   if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
    if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
     if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
      if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
       if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
        if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
 if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
  if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
   if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
    if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
     if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
      if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
       if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
        if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
 if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
  if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
   if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
    if row = 2 and tank = 7 and position = 2 then tankn = '7B2';
     if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
      if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
       if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
        if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
 if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
  if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
   if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
    if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
     if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
      if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
       if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
        if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
 if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
  if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
   if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
```

AEH-12-PSEUDO-04

```
     if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
      if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
       if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
        if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
         if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;
proc sort data= glochidiadist;
 by round x;
 run;
proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artif
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Lake Carlos/whole water tank treatment';
run;
```

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```
*  date created  : AUGUST 11, 2012 - JAL
*  Verified by:  ______ (Date:______)                     page ____ of ____
*  Random allocation of trays to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalic options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1,
2, or 3, etc)
   round = distribution round, place one tray in the assigned position (9 per test
replicate - 3 for each exposure duration) */

/****************************************************************************************
***********/

/*Location and exposure type: Lake Carlos - Whole tank treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 81 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
```

AEH-12-PSEUDO-04

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```
if row = 2 and tank = 2 and position = 1 then tankn = '2B1 ;
 if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
  if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
   if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
    if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
 .   if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
 if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
  if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
   if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
    if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
     if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
      if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
       if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
        if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
 if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
  if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
   if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
    if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
     if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
      if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
       if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
        if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
 if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
  if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
   if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
    if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
     if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
      if row = 3 and tank = 5 and position = 1 then tankn =  5C1';
       if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
        if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
 if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
  if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
   if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
    if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
     if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
      if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
       if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
        if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
 if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
  if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
   if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
    if row = 2 and tank = 7 and position = 2 then tankn = '7B2';
     if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
      if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
       if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
        if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
 if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
  if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
   if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
    if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
```

```
       if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
        if row = 3 and tank = 8 and position = 1 then tankn = '8C1 ;
         if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
          if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
 if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
  if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
   if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
    if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
     if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
      if row = 2 and tank = 9 and position = 3 ther tankn = '9B3';
       if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
        if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
         if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.07 seconds
      cpu time            0.07 seconds


proc sort data= glochidiadist;
 by round x;
 run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra
mussels on artifical substrates';
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Lake Carlos/whole water tank treatment';
run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

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FF # 9a
Item No. 2
Pg 8 of 8

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 1 *** 8/13/12
Lake Carlos - Whole water Body Treatment

JA

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 2 | 0.02216 | 1B2 | 6h |
| 2 | 1 | 3 | 0.16367 | 1A3 | 6h |
| 3 | 1 | 2 | 0.21372 | 1A2 | 6h |
| 4 | 3 | 1 | 0.27213 | 1C1 | 9h |
| 5 | 1 | 1 | 0.29050 | 1A1 | 9h |
| 6 | 2 | 1 | 0.60999 | 1B1 | 9h |
| 7 | 3 | 3 | 0.61991 | 1C3 | 12 |
| 8 | 3 | 2 | 0.95789 | 1C2 | 12 |
| 9 | 2 | 3 | 0.95946 | 1B3 | 12 |

File Folder: 9a ~~0064~~ Item Number: 3 Page 1 of ~~17~~ 30 wrong page #d fix 8APR

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 2 ***
Lake Carlos - Whole water Body Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 2 | 0.05116 | 2B2 | 6h |
| 2 | 1 | 1 | 0.15754 | 2A1 | 6h |
| 3 | 3 | 1 | 0.19038 | 2C1 | 6h |
| 4 | 1 | 3 | 0.29436 | 2A3 | 9h |
| 5 | 3 | 3 | 0.36230 | 2C3 | 9h |
| 6 | 2 | 3 | 0.63280 | 2B3 | 9h |
| 7 | 3 | 2 | 0.73826 | 2C2 | 12 |
| 8 | 1 | 2 | 0.86034 | 2A2 | 12 |
| 9 | 2 | 1 | 0.87946 | 2B1 | 12 |

Page ____ of ____ Item Number

Page 2 of ~~6~~ 30 wrong page # JMW 28AUG12

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 3 ***
Lake Carlos - Whole water Body Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 3 | 3 | 0.09037 | 3C3 | 6h |
| 2 | 1 | 1 | 0.20055 | 3A1 | 6h |
| 3 | 2 | 1 | 0.37245 | 3B1 | 6h |
| 4 | 3 | 2 | 0.38436 | 3C2 | 9h |
| 5 | 1 | 3 | 0.41454 | 3A3 | 9h |
| 6 | 2 | 3 | 0.54343 | 3B3 | 9h |
| 7 | 3 | 1 | 0.54506 | 3C1 | 12 |
| 8 | 2 | 2 | 0.65481 | 3B2 | 12 |
| 9 | 1 | 2 | 0.86888 | 3A2 | 12 |

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Page 3 of ~~17~~ 30 wrong page # KLD 28Mar14

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 4 ***
Lake Carlos - Whole water Body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 3 | 1 | 0.10801 | 4C1 | 6h |
| 2 | 3 | 2 | 0.11324 | 4C2 | 6h |
| 3 | 2 | 2 | 0.23268 | 4B2 | 6h |
| 4 | 1 | 3 | 0.29166 | 4A3 | 9h |
| 5 | 1 | 1 | 0.61787 | 4A1 | 9h |
| 6 | 2 | 3 | 0.68056 | 4B3 | 9h |
| 7 | 3 | 3 | 0.87818 | 4C3 | 12 |
| 8 | 2 | 1 | 0.87898 | 4B1 | 12 |
| 9 | 1 | 2 | 0.99026 | 4A2 | 12 |

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 5 ***
Lake Carlos - Whole water Body Treatment

| Obs | row | position | x | ① tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 2 | 0.05116 | 2B2 | 6h |
| 2 | 1 | 1 | 0.15754 | 2A1 | 6h |
| 3 | 3 | 1 | 0.19038 | 2C1 | 6h |
| 4 | 1 | 3 | 0.29436 | 2A3 | 9h |
| 5 | 3 | 3 | 0.36230 | 2C3 | 9h |
| 6 | 2 | 3 | 0.63280 | 2B3 | 9h |
| 7 | 3 | 2 | 0.73826 | 2C2 | 12 |
| 8 | 1 | 2 | 0.86034 | 2A2 | 12 |
| 9 | 2 | 1 | 0.87946 | 2B1 | 12 |

① Tank numbers should be 5 not 2. KW 15AUG12
See Deviation #2 for further clarification. KW 19NOV13

Page ___ of ___

Page 5 of ~~17~~ 30 wrong page # KW 28APR14

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 6 ***
Lake Carlos - Whole water Body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.13967 | 6A3 | 6h |
| 2 | 3 | 3 | 0.18498 | 6C3 | 6h |
| 3 | 1 | 2 | 0.22522 | 6A2 | 6h |
| 4 | 3 | 1 | 0.29669 | 6C1 | 9h |
| 5 | 2 | 3 | 0.43557 | 6B3 | 9h |
| 6 | 2 | 2 | 0.50443 | 6B2 | 9h |
| 7 | 3 | 2 | 0.69851 | 6C2 | 12 |
| 8 | 1 | 1 | 0.76815 | 6A1 | 12 |
| 9 | 2 | 1 | 0.83108 | 6B1 | 12 |

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Page 6 of ~~17~~ 30 Wrong page # KLW 28APR14

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 7 ***
Lake Carlos - Whole water Body Treatment

| Obs | row | position | x | (1) tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 2 | 0.05116 | 2B2 | 6h |
| 2 | 1 | 1 | 0.15754 | 2A1 | 6h |
| 3 | 3 | 1 | 0.19038 | 2C1 | 6h |
| 4 | 1 | 3 | 0.29436 | 2A3 | 9h |
| 5 | 3 | 3 | 0.36230 | 2C3 | 9h |
| 6 | 2 | 3 | 0.63280 | 2B3 | 9h |
| 7 | 3 | 2 | 0.73826 | 2C2 | 12 |
| 8 | 1 | 2 | 0.86034 | 2A2 | 12 |
| 9 | 2 | 1 | 0.87946 | 2B1 | 12 |

(1) Tank number should be 7 not 2. KW 15AUG13
See Deviation #2 for further clarification. KW 19NOV13

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 8 ***
Lake Carlos - Whole water Body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 0.21368 | 8B3 | 6h |
| 2 | 2 | 2 | 0.23698 | 8B2 | 6h |
| 3 | 2 | 1 | 0.25634 | 8B1 | 6h |
| 4 | 1 | 3 | 0.47516 | 8A3 | 9h |
| 5 | 3 | 1 | 0.61003 | 8C1 | 9h |
| 6 | 3 | 3 | 0.61436 | 8C3 | 9h |
| 7 | 1 | 2 | 0.66954 | 8A2 | 12 |
| 8 | 3 | 2 | 0.93075 | 8C2 | 12 |
| 9 | 1 | 1 | 0.93965 | 8A1 | 12 |

wrong page # KPW 28APR14

AEH-12-PSEUDO-04

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 Sn L

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 9 ***
Lake Carlos - Whole water Body Treatment

| Obs | row | position | x | (1)tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 2 | 0.05116 | 2B2 | 6h |
| 2 | 1 | 1 | 0.15764 | 2A1 | 6h |
| 3 | 3 | 1 | 0.19038 | 2C1 | 6h |
| 4 | 1 | 3 | 0.29436 | 2A3 | 9h |
| 5 | 3 | 3 | 0.36230 | 2C3 | 9h |
| 6 | 2 | 3 | 0.63280 | 2B3 | 9h |
| 7 | 3 | 2 | 0.73826 | 2C2 | 12 |
| 8 | 1 | 2 | 0.86034 | 2A2 | 12 |
| 9 | 2 | 1 | 0.87946 | 2B1 | 12 |

(1) Tank number should be 9 not 2. Km 15AUG12
See Deviation #2 for further clarification. PMW 19NOV13

Page 9 of 173 wrong page # ... 28APR1

Analysis performed by J. Luoma SAS version  9.2 10:20 13AUG12

```
/*****************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 13 August 2012 - JAL
*  Verified by:  _______ (Date:_______)                page ____ of ____
*  Random allocation of treatment to tank.sas
*****************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=105 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
                            Substrate removal from tanks. See title below. KWW 28APR14
/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Lake Carlos - whole tank treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
     run;
proc sort data=TANK1A;
 by x;
run;

data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
```

AEH-12-PSEUDO-04

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AEH-12-PSEUDO-04

```
data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
    if row = 2 and position = 1 then tankn = '2B1';
     if row = 2 and position = 2 then tankn = '2B2';
      if row = 2 and position = 3 then tankn = '2B3';
       if row = 3 and position = 1 then tankn = '2C1';
        if row = 3 and position = 2 then tankn = '2C2';
         if row = 3 and position = 3 then tankn = '2C3';
     run;
proc sort data=TANK2A;
 by x;
run;

data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
```

```
        if row = 3 and position = 1 then tankn = '3C1';
         if row = 3 and position = 2 then tankn = '3C2';
          if row = 3 and position = 3 then tankn = '3C3';
      run;
proc sort data=TANK3A;
 by x;
run;

data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;
proc sort data=TANK4A;
 by x;
run;

data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
```

```
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK5A; set TANK5;
 if row = 1 and position = 1 then tankn = '5A1';
  if row = 1 and position = 2 then tankn = '5A2';
   if row = 1 and position = 3 then tankn = '5A3';
    if row = 2 and position = 1 then tankn = '5B1';
     if row = 2 and position = 2 then tankn = '5B2';
      if row = 2 and position = 3 then tankn = '5B3';
       if row = 3 and position = 1 then tankn = '5C1';
        if row = 3 and position = 2 then tankn = '5C2';
         if row = 3 and position = 3 then tankn = '5C3';
     run;
proc sort data=TANK5A;
 by x;
run;

data assign_trt_TANK5A; set TANK5A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
```

```
run;
data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;
proc sort data=TANK6A;
 by x;
run;

data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn = '7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;
proc sort data=TANK7A;
 by x;
run;
```

```
data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h ;
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;
proc sort data=TANK8A;
 by x;
run;

data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
```

AEH-12-PSEUDO-04

```
run;
data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
     run;
proc sort data=TANK9A;
 by x;
run;

data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
 if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;
```

8/13/12
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```
* date created  : 13 August 2012 - JAL JAL
* Verified by:  _______ (Date:_______)                     page ____ of ____
* Random allocation of treatment to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=105 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
                                   Substrate removal from tanks. See title on next page. 28 APR 14
/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Lake Carlos - whole tank treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK1 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK1.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK1A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-12-PSEUDO-04

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```
data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.ASSIGN_TRT_TANK1A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK1A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.09 seconds
      cpu time            0.03 seconds


data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK2 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds
```



```
data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
```

```
    if row = 2 and position = 1 then tankn = '2B1';
     if row = 2 and position = 2 then tankn = '2B2';
      if row = 2 and position = 3 then tankn = '2B3';
       if row = 3 and position = 1 then tankn = '2C1';
        if row = 3 and position = 2 then tankn = '2C2';
         if row = 3 and position = 3 then tankn = '2C3 ;
     run;

NOTE: There were 9 observations read from the data set WORK.TANK2.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK2A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds



data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.ASSIGN_TRT_TANK2A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignmont of substrate renoval from tanks *** TANK 2 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
```

```
     real time           0.00 seconds
     cpu time            0.01 seconds


data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK3 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
       if row = 3 and position = 1 then tankn = '3C1';
        if row = 3 and position = 2 then tankn = '3C2';
         if row = 3 and position = 3 then tankn = '3C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK3.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc sort data=TANK3A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
```

```
if _n_ = 5 then trt = '9h';
 if _n_ = 6 then trt = '9h';
  if _n_ = 7 then trt = '12h';
   if _n_ = 8 then trt = '12h';
if _n_ = 9 then trt = '12h';
 run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.ASSIGN_TRT_TANK3A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas flucrescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK3A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK4 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK4.
```

```
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

AEH-12-PSEUDO-04

```
proc sort data=TANK4A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.05 seconds
      cpu time            0.00 seconds


data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.ASSIGN_TRT_TANK4A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK4A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
```

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```
  end;
 end;
run;

NOTE: The data set WORK.TANK5 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds

data TANK5A; set TANK5;
 if row = 1 and position = 1 then tankn = '5A1';
  if row = 1 and position = 2 then tankn = '5A2';
   if row = 1 and position = 3 then tankn = '5A3';
    if row = 2 and position = 1 then tankn = '5B1';
     if row = 2 and position = 2 then tankn = '5B2';
      if row = 2 and position = 3 then tankn = '5B3';
       if row = 3 and position = 1 then tankn = '5C1';
        if row = 3 and position = 2 then tankn = '5C2';
         if row = 3 and position = 3 then tankn = '5C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK5.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds

proc sort data=TANK5A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds

data assign_trt_TANK5A; set TANK5A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.ASSIGN_TRT_TANK5A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
```

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```
      real time           0.00 seconds
      cpu time            0.01 seconds

proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK6 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK6.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc sort data=TANK6A;
 by x;
run;
```

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```
NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


 
 data assign_trt_TANK6A; set TANK6A;
  if _n_ = 1 then trt = '6h';
   if _n_ = 2 then trt = '6h';
    if _n_ = 3 then trt = '6h';
     if _n_ = 4 then trt = '9h';
  if _n_ = 5 then trt = '9h';
   if _n_ = 6 then trt = '9h';
    if _n_ = 7 then trt = '12h';
     if _n_ = 8 then trt = '12h';
  if _n_ = 9 then trt = '12h';
   run;

NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.ASSIGN_TRT_TANK6A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


 proc print data= assign_trt_TANK6A;
 title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
 artifical substrates';
 title2 h=1.5 'AEH-12-PSEUDO-04';
 title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
 title4 h=1 'Lake Carlos - Whole water Body Treatment  ;
 run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK6A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


 data TANK7;
 do row = 1 to 3 by 1;
  do position = 1 to 3 by 1;
   x = ranuni(-1);
   output;
   end;
  end;
 run;

NOTE: The data set WORK.TANK7 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

```
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn = '7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK7.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc sort data=TANK7A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.00 seconds



data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.ASSIGN_TRT_TANK7A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds
```



```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
```

```
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

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```
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK8 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK8.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc sort data=TANK8A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds
```



```
data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
```

AEH-12-PSEUDO-04

```
 if _n_ = 2 then trt = '6h';
  if _n_ = 3 then trt = '6h';
   if _n_ = 4 then trt = '9h';
if _n_ = 5 then trt = '9h';
 if _n_ = 6 then trt = '9h';
  if _n_ = 7 then trt = '12h';
   if _n_ = 8 then trt = '12h';
if _n_ = 9 then trt = '12h';
 run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.ASSIGN_TRT_TANK8A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK8A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK9 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
```

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```
      run;

NOTE: There were 9 observations read from the data set WORK.TANK9.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

AEH-12-PSEUDO-04

```
proc sort data=TANK9A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.ASSIGN_TRT_TANK9A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Lake Carlos - Whole water Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

FF # 9a
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8/13/12 JO

```
NOTE: This SAS session is using a registry in WORK.  All changes will be lost at the end of this
      session.
```

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrat 1
AEH-12-PSEUDO-04
Random assignment of treatment to experimental tanks 8/11/12
Treatment Location/type: Lake Carlos - bottom injection JL

AEH-12-PSEUDO-04

| Obs | block | tank | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 6 | 0.13335 | Tank 6 | control |
| 2 | 1 | 1 | 0.20575 | Tank 1 | 50 |
| 3 | 1 | 5 | 0.28506 | Tank 5 | 100 |
| 4 | 1 | 7 | 0.34557 | Tank 7 | control |
| 5 | 1 | 4 | 0.34624 | Tank 4 | 50 |
| 6 | 1 | 2 | 0.55080 | Tank 2 | 100 |
| 7 | 1 | 3 | 0.59072 | Tank 3 | control |
| 8 | 1 | 8 | 0.70738 | Tank 8 | 50 |
| 9 | 1 | 9 | 0.88671 | Tank 9 | 100 |

File Folder: 12a Item Number: 1 Page 1 of 4

Analysis performed by J. Luoma SAS version 9.2 08:59 11AUG12

```
/*******************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 11 August 2012 - JAL JM
*  Verified by:  _______ (Date:_______)                    page ____ of ____   AEH-12-PSEUDO-04
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Lake Carlos - bottom injection exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;
proc sort data=fish2;
 by block x;
run;

data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;
proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Lake Carlos - bottom injection';
run;
```

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```
*  date created  : 11 August 2012 - JAL
*  Verified by:  ______  (Date:______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Lake Carlos - bottom injection exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.FISH has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
    run;

NOTE: There were 9 observations read from the data set WORK.FISH.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=fish2;
 by block x;
run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

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```
data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.ASSIGN_TRT_FISH has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Lake Carlos - bottom injection';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_FISH.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

8/11/12

Jn

**FF #** 12A
**Item No.** 1
**Pg** 4 **of** 4

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical subs 1
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position 8/11/12 JL
Test Location/type = Lake Carlos/Bottom injection tank treatment

AEH-12-PSEUDO-04

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 3 | 6 | 0.01486 | A | 6A3 |
| 2 | 1 | 1 | 3 | 8 | 0.02061 | A | 8A3 |
| 3 | 1 | 3 | 1 | 9 | 0.02926 | C | 9C1 |
| 4 | 1 | 3 | 1 | 5 | 0.03436 | C | 5C1 |
| 5 | 1 | 3 | 1 | 6 | 0.04324 | C | 6C1 |
| 6 | 1 | 2 | 2 | 9 | 0.06133 | B | 9B2 |
| 7 | 1 | 1 | 3 | 1 | 0.08194 | A | 1A3 |
| 8 | 1 | 3 | 1 | 1 | 0.10063 | C | 1C1 |
| 9 | 1 | 3 | 3 | 8 | 0.10670 | C | 8C3 |
| 10 | 1 | 2 | 2 | 6 | 0.10789 | B | 6B2 |
| 11 | 1 | 3 | 3 | 5 | 0.12424 | C | 5C3 |
| 12 | 1 | 3 | 2 | 5 | 0.12608 | C | 5C2 |
| 13 | 1 | 3 | 3 | 3 | 0.13019 | C | 3C3 |
| 14 | 1 | 3 | 3 | 7 | 0.15140 | C | 7C3 |
| 15 | 1 | 1 | 2 | 1 | 0.15824 | A | 1A2 |
| 16 | 1 | 2 | 1 | 6 | 0.17372 | B | 6B1 |
| 17 | 1 | 2 | 2 | 7 | 0.18856 | B | 7B2 |
| 18 | 1 | 3 | 3 | 6 | 0.20423 | C | 6C3 |
| 19 | 1 | 2 | 1 | 2 | 0.21778 | B | 2B1 |
| 20 | 1 | 2 | 1 | 9 | 0.22759 | B | 9B1 |
| 21 | 1 | 2 | 3 | 5 | 0.23906 | B | 5B3 |
| 22 | 1 | 1 | 3 | 5 | 0.24018 | A | 5A3 |
| 23 | 1 | 3 | 1 | 8 | 0.26362 | C | 8C1 |
| 24 | 1 | 2 | 3 | 3 | 0.27612 | B | 3B3 |
| 25 | 1 | 3 | 3 | 1 | 0.28044 | C | 1C3 |
| 26 | 1 | 2 | 2 | 2 | 0.30348 | B | 2B2 |
| 27 | 1 | 1 | 1 | 4 | 0.33272 | A | 4A1 |
| 28 | 1 | 1 | 1 | 8 | 0.34190 | A | 8A1 |
| 29 | 1 | 1 | 3 | 4 | 0.35625 | A | 4A3 |
| 30 | 1 | 3 | 3 | 2 | 0.35832 | C | 2C3 |
| 31 | 1 | 2 | 3 | 9 | 0.36298 | B | 9B3 |
| 32 | 1 | 1 | 2 | 3 | 0.37363 | A | 3A2 |
| 33 | 1 | 1 | 1 | 5 | 0.39157 | A | 5A1 |
| 34 | 1 | 2 | 2 | 4 | 0.39863 | B | 4B2 |
| 35 | 1 | 1 | 2 | 2 | 0.44104 | A | 2A2 |
| 36 | 1 | 2 | 2 | 1 | 0.44969 | B | 1B2 |
| 37 | 1 | 3 | 3 | 9 | 0.47858 | C | 9C3 |
| 38 | 1 | 1 | 3 | 3 | 0.47917 | A | 3A3 |
| 39 | 1 | 1 | 2 | 6 | 0.48418 | A | 6A2 |
| 40 | 1 | 3 | 1 | 4 | 0.49023 | C | 4C1 |
| 41 | 1 | 1 | 2 | 7 | 0.49744 | A | 7A2 |
| 42 | 1 | 1 | 2 | 9 | 0.49994 | A | 9A2 |
| 43 | 1 | 3 | 1 | 3 | 0.52922 | C | 3C1 |
| 44 | 1 | 2 | 3 | 1 | 0.53076 | B | 1B3 |
| 45 | 1 | 1 | 1 | 7 | 0.54877 | A | 7A1 |
| 46 | 1 | 2 | 3 | 7 | 0.59766 | B | 7B3 |
| 47 | 1 | 2 | 1 | 8 | 0.60780 | B | 8B1 |

Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12

✓ indicates bag positions to be used for the 12 h treatment
KW
16AUG12

Began distribution @ 1050

Finish distribution @ 1106

File Folder: 12a

Item Number: 2

Page 1 of 108 wrong page # KW 28AUG12

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical subs 2
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Lake Carlos/Bottom injection tank treatment

AEH-12-PSEUDO-04

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 48 | 1 | 2 | 3 | 6 | 0.61277 | B | 6B3 |
| 49 | 1 | 3 | 2 | 8 | 0.61554 | C | 8C2 |
| 50 | 1 | 1 | 3 | 2 | 0.63169 | A | 2A3 |
| 51 | 1 | 2 | 3 | 8 | 0.63320 | B | 8B3 |
| 52 | 1 | 3 | 2 | 6 | 0.64911 | C | 6C2 |
| 53 | 1 | 1 | 1 | 3 | 0.65102 | A | 3A1 |
| 54 | 1 | 2 | 1 | 5 | 0.65765 | B | 5B1 |
| 55 | 1 | 2 | 3 | 4 | 0.66704 | B | 4B3 |
| 56 | 1 | 3 | 3 | 4 | 0.67056 | C | 4C3 |
| 57 | 1 | 1 | 2 | 8 | 0.67307 | A | 8A2 |
| 58 | 1 | 1 | 2 | 4 | 0.69925 | A | 4A2 |
| 59 | 1 | 1 | 3 | 7 | 0.71438 | A | 7A3 |
| 60 | 1 | 3 | 2 | 7 | 0.71487 | C | 7C2 |
| 61 | 1 | 2 | 2 | 8 | 0.71755 | B | 8B2 |
| 62 | 1 | 1 | 3 | 9 | 0.71982 | A | 9A3 |
| 63 | 1 | 3 | 2 | 3 | 0.74468 | C | 3C2 |
| 64 | 1 | 1 | 1 | 1 | 0.78165 | A | 1A1 |
| 65 | 1 | 3 | 1 | 7 | 0.78878 | C | 7C1 |
| 66 | 1 | 1 | 1 | 6 | 0.80236 | A | 6A1 |
| 67 | 1 | 3 | 2 | 4 | 0.80989 | C | 4C2 |
| 68 | 1 | 2 | 1 | 7 | 0.81173 | B | 7B1 |
| 69 | 1 | 1 | 2 | 5 | 0.81727 | A | 5A2 |
| 70 | 1 | 2 | 1 | 4 | 0.81863 | B | 4B1 |
| 71 | 1 | 1 | 1 | 9 | 0.86794 | A | 9A1 |
| 72 | 1 | 1 | 1 | 2 | 0.87148 | A | 2A1 |
| 73 | 1 | 3 | 2 | 2 | 0.87686 | C | 2C2 |
| 74 | 1 | 2 | 2 | 3 | 0.89412 | B | 3B2 |
| 75 | 1 | 2 | 3 | 2 | 0.89639 | B | 2B3 |
| 76 | 1 | 3 | 1 | 2 | 0.91321 | C | 2C1 |
| 77 | 1 | 2 | 1 | 3 | 0.93071 | B | 3B1 |
| 78 | 1 | 3 | 2 | 9 | 0.94301 | C | 9C2 |
| 79 | 1 | 2 | 2 | 5 | 0.94979 | B | 5B2 |
| 80 | 1 | 2 | 1 | 1 | 0.95449 | B | 1B1 |
| 81 | 1 | 3 | 2 | 1 | 0.98806 | C | 1C2 |

Page 2 of 108 wrong page# find error

Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12 JL

```
/*****************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : AUGUST 11, 2012 - JAL
*  Verified by:  _______ (Date:_______)              page ____ of ____
*  Random allocation of trays to tank.sas
*****************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2, or 3, e
   round = distribution round, place one tray in the assigned position (9 per test replicate - 3 for

/*****************************************************************************************************

/*Location and exposure type: Lake Carlos - Bottom injection treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1 ;
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
 if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
  if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
```

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AEH-12-PSEUDO-04

```
  if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
   if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
    if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
     if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
      if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
       if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
        if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
 if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
  if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
   if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
    if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
     if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
      if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
       if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
        if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
 if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
  if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
   if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
    if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
     if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
      if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
       if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
        if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
 if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
  if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
   if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
    if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
     if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
      if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
       if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
        if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
 if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
  if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
   if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
    if row = 2 and tank = 7 and position = 2 then tankn = '7B2';
     if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
      if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
       if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
        if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
 if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
  if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
   if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
    if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
     if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
      if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
       if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
        if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
 if row = 1 and tank = 9 and oosition = 2 then tankn = '9A2';
  if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
   if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
```

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```
     if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
      if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
       if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
        if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
         if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;
proc sort data= glochidiadist;
 by round x;
 run;
proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artif
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Lake Carlos/Bottom injection tank treatment';
run;
```

AEH-12-PSEUDO-04

8/11/12
JA

```
*  date created  : AUGUST 11, 2012 - JAL
*  Verified by:  ______ (Date:______)  JAW                    page ____ of ____
*  Random allocation of trays to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2,
or 3, etc)
   round = distribution round, place one tray in the assigned position (9 per test replicate -
 3 for each exposure duration) */

/*********************************************************************************************
********/

/*Location and exposure type: Lake Carlos - Bottom injection treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 81 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn =  1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
```

AEH-12-PSEUDO-04

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```
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
 if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
  if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
   if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
    if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
     if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
      if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
       if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
        if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
 if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
  if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
   if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
    if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
     if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
      if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
       if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
        if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
 if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
  if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
   if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
    if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
     if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
      if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
       if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
        if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
 if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
  if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
   if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
    if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
     if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
      if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
       if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
        if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
 if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
  if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
   if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
    if row = 2 and tank = 7 and position = 2 then tankn = '7B2';
     if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
      if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
       if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
        if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
 if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
  if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
   if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
    if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
```

```
    if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
     if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
      if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
       if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
 if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
  if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
   if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
    if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
     if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
      if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
       if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
        if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;
```

AEH-12-PSEUDO-04

```
NOTE: There were 81 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.07 seconds
      cpu time            0.07 seconds


proc sort data= glochidiadist;
 by round x;
 run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels
on artifical substrates';
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Lake Carlos/Bottom injection tank treatment';
run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

8/11/16
JS

FF # 12a
Item No. 2
Pg 8 of 8

:fficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 1 ***
Lake Carlos - bottom injection Treatment

8/13/12 AEH-12-PSEUDO-04
JL

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| ~~1~~ | ~~3~~ | ~~2~~ | ~~0.00578~~ | ~~1C2~~ | ~~6h~~ |
| ~~2~~ | ~~3~~ | ~~3~~ | ~~0.07326~~ | ~~1C3~~ | ~~6h~~ |
| ~~3~~ | ~~3~~ | ~~1~~ | ~~0.24288~~ | ~~1C1~~ | ~~6h~~ |
| ~~4~~ | ~~1~~ | ~~2~~ | ~~0.27838~~ | ~~1A2~~ | ~~9h~~ |
| ~~5~~ | ~~1~~ | ~~3~~ | ~~0.28017~~ | ~~1A3~~ | ~~9h~~ |
| ~~6~~ | ~~2~~ | ~~1~~ | ~~0.53070~~ | ~~1B1~~ | ~~9h~~ |
| 7 | 2 | 2 | 0.61638 | 1B2 | 12 |
| 8 | 2 | 3 | 0.68756 | 1B3 | 12 |
| 9 | 1 | 1 | 0.81146 | 1A1 | 12 |

Dosing for bottom injection is occurring for 12 h with no 6 or 9 h sampling.
JLW 16AUG12

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AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 2 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 0.11803 | 2A2 | 6h |
| 2 | 2 | 2 | 0.62053 | 2B2 | 6h |
| 3 | 2 | 1 | 0.71937 | 2B1 | 6h |
| 4 | 1 | 3 | 0.87192 | 2A3 | 9h |
| 5 | 3 | 2 | 0.91454 | 2C2 | 9h |
| 6 | 3 | 3 | 0.94135 | 2C3 | 9h |
| 7 | 2 | 3 | 0.95412 | 2B3 | 12 |
| 8 | 1 | 1 | 0.97236 | 2A1 | 12 |
| 9 | 3 | 1 | 0.99390 | 2C1 | 12 |

Dosing for bottom Injection is occurring for 12 h with no 6 or 9 h sampling.
JLW
16AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 3 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 3 | 2 | 0.19914 | 3C2 | 6h |
| 2 | 1 | 2 | 0.28840 | 3A2 | 6h |
| 3 | 2 | 3 | 0.38093 | 3B3 | 6h |
| 4 | 3 | 1 | 0.43250 | 3C1 | 9h |
| 5 | 1 | 1 | 0.57180 | 3A1 | 9h |
| 6 | 2 | 2 | 0.66134 | 3B2 | 9h |
| 7 | 3 | 3 | 0.71983 | 3C3 | 12 |
| 8 | 1 | 3 | 0.82826 | 3A3 | 12 |
| 9 | 2 | 1 | 0.88773 | 3B1 | 12 |

Dosing for bottom injection is occurring for 12 h with no 6 or 9 h sampling.
KW 16AUG12

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Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 4 ***
Lake Carlos - bottom injection Body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 0.04712 | 4A2 | 6h |
| 2 | 3 | 3 | 0.06272 | 4C3 | 6h |
| 3 | 3 | 1 | 0.14155 | 4C1 | 6h |
| 4 | 3 | 2 | 0.28865 | 4C2 | 9h |
| 5 | 1 | 1 | 0.45448 | 4A1 | 9h |
| 6 | 1 | 3 | 0.50193 | 4A3 | 9h |
| 7 | 2 | 1 | 0.73873 | 4B1 | 12 |
| 8 | 2 | 3 | 0.88940 | 4B3 | 12 |
| 9 | 2 | 2 | 0.94491 | 4B2 | 12 |

Dosing for bottom injection is occurring for 12 h with no 6 or 9 h sampling.
KW
16AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 5 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | ①tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 0.11803 | 2A2 | 6h |
| 2 | 2 | 2 | 0.62053 | 2B2 | 6h |
| 3 | 2 | 1 | 0.71937 | 2B1 | 6h |
| 4 | 1 | 3 | 0.87192 | 2A3 | 9h |
| 5 | 3 | 2 | 0.91454 | 2C2 | 9h |
| 6 | 3 | 3 | 0.94135 | 2C3 | 9h |
| 7 | 2 | 3 | 0.95412 | 2B3 | 12 |
| 8 | 1 | 1 | 0.97236 | 2A1 | 12 |
| 9 | 3 | 1 | 0.99390 | 2C1 | 12 |

Dosing for bottom injection is occurring for 12 h with no 6 or 9 h sampling. KW 16AUG12

① Tank ID should be 5 not 2 KW 16AUG12
See Deviation #2 for further clarification. KW 1JAN013

AEH-12-PSEUDO-04

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 6 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 3 | 2 | 0.08423 | 6C2 | 6h |
| 2 | 3 | 3 | 0.16043 | 6C3 | 6h |
| 3 | 1 | 3 | 0.20065 | 6A3 | 6h |
| 4 | 1 | 1 | 0.29470 | 6A1 | 9h |
| 5 | 2 | 2 | 0.30688 | 6B2 | 9h |
| 6 | 2 | 3 | 0.35165 | 6B3 | 9h |
| 7 | 1 | 2 | 0.38714 | 6A2 | 12 |
| 8 | 3 | 1 | 0.60130 | 6C1 | 12 |
| 9 | 2 | 1 | 0.64342 | 6B1 | 12 |

Dosing for bottom injection is for 12 h with no 6 or 9 h sampling. KW 16AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 7 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | ①tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 0.11803 | 2A2 | 6h |
| 2 | 2 | 2 | 0.62053 | 2B2 | 6h |
| 3 | 2 | 1 | 0.71937 | 2B1 | 6h |
| 4 | 1 | 3 | 0.67192 | 2A3 | 9h |
| 5 | 3 | 2 | 0.91454 | 2C2 | 9h |
| 6 | 3 | 3 | 0.94135 | 2C3 | 9h |
| 7 | 2 | 3 | 0.95412 | 2B3 | 12 |
| 8 | 1 | 1 | 0.97236 | 2A1 | 12 |
| 9 | 3 | 1 | 0.99390 | 2C1 | 12 |

Dosing for bottom injection 13 for 12 h with no 6 or 9 h Sampling. KW 16AUG12

① Tank ID should be 7 not 2. KW 16AUG12
See Deviation #2 for further clarification. KW 19NOV13

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Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 8 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 3 | 1 | 0.06104 | 8C1 | 6h |
| 2 | 1 | 1 | 0.13492 | 8A1 | 6h |
| 3 | 3 | 3 | 0.25814 | 8C3 | 6h |
| 4 | 2 | 1 | 0.54811 | 8B1 | 9h |
| 5 | 2 | 2 | 0.66886 | 8B2 | 9h |
| 6 | 1 | 2 | 0.72715 | 8A2 | 9h |
| 7 | 2 | 3 | 0.81389 | 8B3 | 12 |
| 8 | 3 | 2 | 0.87980 | 8C2 | 12 |
| 9 | 1 | 3 | 0.89938 | 8A3 | 12 |

Dosing for bottom injection B for 12 h with no 6 or 9 h sampling KW 16AUG12

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AEH-12-PSEUDO-04

~~AEH-12-PSEUDO-04~~ NL KMW 29APR2014

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 9 ***
Lake Carlos - bottom injection Treatment

| Obs | row | position | x | ① tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 0.11803 | 2A2 | 6h |
| 2 | 2 | 2 | 0.62053 | 2B2 | 6h |
| 3 | 2 | 1 | 0.71937 | 2B1 | 6h |
| 4 | 1 | 3 | 0.87192 | 2A3 | 9h |
| 5 | 3 | 2 | 0.91454 | 2C2 | 9h |
| 6 | 3 | 3 | 0.94135 | 2C3 | 9h |
| 7 | 2 | 3 | 0.95412 | 2B3 | 12 |
| 8 | 1 | 1 | 0.97236 | 2A1 | 12 |
| 9 | 3 | 1 | 0.99390 | 2C1 | 12 |

Dosing for bottom injection is for 12h with no 6 or 9h sampling. KMW 16AUG12

① Tank ID should be 9 not 2. KMW 16AUG12

See Deviation #2 for further clarification. KMW 19NOV13

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AEH-12-PSEUDO-04

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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/***********************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 13 August 2012 - JAL 8/13/12
*  Verified by:  _______ (Date:_______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
***********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=105 ps=64 formdlim='-' pageno = 1 nocenter nodate nosource2;
                            substrate removal from tanks. See title below. KW 25APR14
/*Random assignment of ~~treatment to experimental tanks*~~/
/*Location/exposure type: Lake Carlos - bottom injection treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
     run;
proc sort data=TANK1A;
 by x;
run;

data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;
```

```
data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
    if row = 2 and position = 1 then tankn = '2B1';
     if row = 2 and position = 2 then tankn = '2B2';
      if row = 2 and position = 3 then tankn = '2B3';
       if row = 3 and position = 1 then tankn = '2C1';
        if row = 3 and position = 2 then tankn = '2C2';
         if row = 3 and position = 3 then tankn = '2C3';
     run;
proc sort data=TANK2A;
 by x;
run;

data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
```

```
       if row = 3 and position = 1 then tankn = '3C1';
        if row = 3 and position = 2 then tankn = '3C2';
         if row = 3 and position = 3 then tankn = '3C3';
     run;
proc sort data=TANK3A;
 by x;
run;

data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _r_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;
data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;
proc sort data=TANK4A;
 by x;
run;

data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
```

```
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04 ;
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Lake Carlos - bottom injection Body Treatment ';
run;
data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK5A; set TANK5;
 if row = 1 and position = 1 then tankn = '5A1';
  if row = 1 and position = 2 then tankn = '5A2';
   if row = 1 and position = 3 then tankn = '5A3';
    if row = 2 and position = 1 then tankn = '5B1';
     if row = 2 and position = 2 then tankn = '5B2';
      if row = 2 and position = 3 then tankn = '5B3';
       if row = 3 and position = 1 then tankn = '5C1';
        if row = 3 and position = 2 then tankn = '5C2';
         if row = 3 and position = 3 then tankn = '5C3';
     run;
proc sort data=TANK5A;
 by x;
run;

data assign_trt_TANK5A; set TANK5A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h ;
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
 proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;
data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
```

```
run;
data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;
proc sort data=TANK6A;
 by x;
run;

data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;
data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn = '7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;
proc sort data=TANK7A;
 by x;
run;
```

```
data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;
proc sort data=TANK8A;
 by x;
run;

data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
```

```
run;
data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
     run;
proc sort data=TANK9A;
 by x;
run;

data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h ;
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluoroscens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;
```

8/13/12
JNL

```
*  date created  : 13 August 2012 - JAL
*  Verified by:  ______ (Date:______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=105 ps=54 formdlim='-' pagono = 1 nocenter nodate nosource2;

/*Random assignment of ~~treatment to experimental tanks*/~~
/*Location/exposure type: Lake Carlos - bottom injection treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK1 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
      run;

NOTE: There were 9 observations read from the data set WORK.TANK1.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK1A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEM-12-PSEUDO-04

Substrate removal from tanks. See title on next page. KW 28APR14

```
data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt =  6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data sot WORK.TANK1A.
NOTE: The data set WORK.ASSIGN_TRT_TANK1A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK1A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK2 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
```

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```
     if row = 2 and position = 1 then tankn = '2B1';
      if row = 2 and position = 2 then tankn = '2B2';
       if row = 2 and position = 3 then tankn = '2B3';
        if row = 3 and position = 1 then tankn = '2C1';
         if row = 3 and position = 2 then tankn = '2C2';
          if row = 3 and position = 3 then tankn = '2C3';
      run;

NOTE: There were 9 observations read from the data set WORK.TANK2.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK2A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.ASSIGN_TRT_TANK2A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
! artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
```

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```
     real time           0.00 seconds
     cpu time            0.00 seconds


data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK3 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
       if row = 3 and position = 1 then tankn = '3C1';
        if row = 3 and position = 2 then tankn = '3C2';
         if row = 3 and position = 3 then tankn = '3C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK3.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=TANK3A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt =  6h';
    if _n_ = 4 then trt = '9h';
```

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```
if _n_ = 5 then trt = '9h';
 if _n_ = 6 then trt = '9h';
  if _n_ = 7 then trt = '12h';
   if _n_ = 8 then trt = '12h';
if _n_ = 9 then trt = '12h';
 run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.ASSIGN_TRT_TANK3A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK3A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK4 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK4.
```

```
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```



```
proc sort data=TANK4A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h ;
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.ASSIGN_TRT_TANK4A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Lake Carlos - bottom injection Body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK4A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
```

```
  end;
 end;
run;

NOTE: The data set WORK.TANK5 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


data TANK5A; set TANK5;
 if row = 1 and position = 1 then tankn = '5A1';
  if row = 1 and position = 2 then tankn = '5A2';
   if row = 1 and position = 3 then tankn = '5A3';
    if row = 2 and position = 1 then tankn = '5B1';
     if row = 2 and position = 2 then tankn = '5B2';
      if row = 2 and position = 3 then tankn = '5B3';
       if row = 3 and position = 1 then tankn = '5C1';
        if row = 3 and position = 2 then tankn = '5C2';
         if row = 3 and position = 3 then tankn = '5C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK5.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK5A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



data assign_trt_TANK5A; set TANK5A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.ASSIGN_TRT_TANK5A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
```

```
      real time           0.01 seconds
      cpu time            0.01 seconds
```



```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK6 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK6.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK6A;
 by x;
run;
```

```
NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```



```

data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.ASSIGN_TRT_TANK6A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK6A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK7 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

```
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn =  7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK7.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK7A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.ASSIGN_TRT_TANK7A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```



```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
```

```
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

AEH-12-PSEUDO-04

```
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK8 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn =  8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK8.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=TANK8A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

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```
data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
```

```
 if _n_ = 2 then trt = '6h';
  if _n_ = 3 then trt = '6h';
   if _n_ = 4 then trt = '9h';
if _n_ = 5 then trt = '9h';
 if _n_ = 6 then trt = '9h';
  if _n_ = 7 then trt =  12h';
   if _n_ = 8 then trt = '12h';
if _n_ = 9 then trt = '12h';
 run;
```

AEH-12-PSEUDO-04

```
NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.ASSIGN_TRT_TANK8A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Lake Carlos - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK8A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK9 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
```

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```
733        run;

NOTE: There were 9 observations read from the data set WORK.TANK9.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-12-PSEUDO-04

```
734  proc sort data=TANK9A;
735   by x;
736  run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


737
738  data assign_trt_TANK9A; set TANK9A;
739   if _n_ = 1 then trt = '6h';
740    if _n_ = 2 then trt = '6h';
741     if _n_ = 3 then trt = '6h';
742      if _n_ = 4 then trt = '9h';
743   if _n_ = 5 then trt = '9h';
744    if _n_ = 6 then trt = '9h';
745     if _n_ = 7 then trt = '12h';
746      if _n_ = 8 then trt = '12h';
747   if _n_ = 9 then trt = '12h';
748    run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.ASSIGN_TRT_TANK9A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


749  proc print data= assign_trt_TANK2A;
750  title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
7501 artifical substrates';
751  title2 h=1.5 'AEH-12-PSEUDO-04';
752  title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
753  title4 h=1 'Lake Carlos - bottom injection Treatment ';
754  run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

File Folder: 12a

Item Number: 3

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8/13/12 JR

```
NOTE: This SAS session is using a registry in WORK.  All changes will be lost at the end of this sess:
```

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrat 1
AEH-12-PSEUDO-04
Random assignment of treatment to experimental tanks
Treatment Location/type: Shawano - whole water body

| Obs | block | tank | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 4 | 0.30968 | Tank 4 | control |
| 2 | 1 | 1 | 0.32204 | Tank 1 | 50 |
| 3 | 1 | 2 | 0.36284 | Tank 2 | 100 |
| 4 | 1 | 8 | 0.39961 | Tank 8 | control |
| 5 | 1 | 9 | 0.45330 | Tank 9 | 50 |
| 6 | 1 | 5 | 0.58960 | Tank 5 | 100 |
| 7 | 1 | 6 | 0.60667 | Tank 6 | control |
| 8 | 1 | 7 | 0.66409 | Tank 7 | 50 |
| 9 | 1 | 3 | 0.99233 | Tank 3 | 100 |

AEH-12-PSEUDO-04

Treatments applied to exposure tanks were not as stated. See Deviation #1 for further clarification.
KLW
19 NOV13

File Folder: 11a   Item Number: 1   Page 1 of 4

Analysis performed by J. Luoma SAS version 9.2 08:59 11AUG12 JL

```
/*********************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 11 August 2012 - JAL
*  Verified by:  _______ (Date:_______)                page ____ of ____
*  Random allocation of treatment to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-  */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Shawano - whole tank exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;
proc sort data=fish2;
 by block x;
run;

data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control ;
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;
proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Shawano - whole water body';
run;
```

AEH-12-PSEUDO-04

Page 2 of 4

```
*  date created  : 11 August 2012 - JAL JL
*  Verified by:  _______ (Date:_______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Shawano - whole tank exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.FISH has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;

NOTE: There were 9 observations read from the data set WORK.FISH.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=fish2;
 by block x;
run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-12-PCEUDC-04

FF # 11a
Item No. 7
Pg 3 of 4

AEH-12-PSEUDO-04

```
data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.ASSIGN_TRT_FISH has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Shawano - whole water body';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_FISH.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

8/11/12
Jn-

FF # 11a
Item No. 1
Pg 4 of 4

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical subs 1
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Shawano whole water column tank treatment

8/11/12
JL

AEH-12-PSEUDO-04

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 2 | 0.00522 | A | 2A1 |
| 2 | 1 | 2 | 3 | 3 | 0.00709 | B | 3B3 |
| 3 | 1 | 3 | 1 | 1 | 0.00785 | C | 1C1 |
| 4 | 1 | 3 | 2 | 3 | 0.02229 | C | 3C2 |
| 5 | 1 | 3 | 3 | 1 | 0.02239 | C | 1C3 |
| 6 | 1 | 1 | 3 | 7 | 0.02452 | A | 7A3 |
| 7 | 1 | 2 | 3 | 4 | 0.02735 | B | 4B3 |
| 8 | 1 | 1 | 1 | 4 | 0.02946 | A | 4A1 |
| 9 | 1 | 2 | 3 | 5 | 0.05448 | B | 5B3 |
| 10 | 1 | 1 | 2 | 8 | 0.06361 | A | 8A2 |
| 11 | 1 | 2 | 2 | 1 | 0.06658 | B | 1B2 |
| 12 | 1 | 2 | 1 | 8 | 0.06729 | B | 8B1 |
| 13 | 1 | 3 | 3 | 9 | 0.06782 | C | 9C3 |
| 14 | 1 | 3 | 3 | 6 | 0.06930 | C | 6C3 |
| 15 | 1 | 1 | 2 | 7 | 0.07870 | A | 7A2 |
| 16 | 1 | 2 | 2 | 3 | 0.08066 | B | 3B2 |
| 17 | 1 | 1 | 3 | 3 | 0.08124 | A | 3A3 |
| 18 | 1 | 1 | 3 | 2 | 0.09097 | A | 2A3 |
| 19 | 1 | 3 | 2 | 8 | 0.09676 | C | 8C2 |
| 20 | 1 | 2 | 2 | 7 | 0.11058 | B | 7B2 |
| 21 | 1 | 3 | 3 | 5 | 0.11777 | C | 5C3 |
| 22 | 1 | 3 | 1 | 9 | 0.12717 | C | 9C1 |
| 23 | 1 | 2 | 1 | 3 | 0.13584 | B | 3B1 |
| 24 | 1 | 3 | 2 | 7 | 0.14704 | C | 7C2 |
| 25 | 1 | 3 | 1 | 7 | 0.15021 | C | 7C1 |
| 26 | 1 | 2 | 3 | 2 | 0.15362 | B | 2B3 |
| 27 | 1 | 1 | 3 | 4 | 0.16437 | A | 4A3 |
| 28 | 1 | 1 | 2 | 3 | 0.19665 | A | 3A2 |
| 29 | 1 | 2 | 3 | 9 | 0.21558 | B | 9B3 |
| 30 | 1 | 3 | 2 | 4 | 0.22984 | C | 4C2 |
| 31 | 1 | 2 | 1 | 7 | 0.26785 | B | 7B1 |
| 32 | 1 | 2 | 2 | 5 | 0.27061 | B | 5B2 |
| 33 | 1 | 3 | 1 | 2 | 0.27844 | C | 2C1 |
| 34 | 1 | 3 | 3 | 4 | 0.29757 | C | 4C3 |
| 35 | 1 | 2 | 2 | 4 | 0.32852 | B | 4B2 |
| 36 | 1 | 1 | 2 | 5 | 0.33047 | A | 5A2 |
| 37 | 1 | 3 | 3 | 2 | 0.36000 | C | 2C3 |
| 38 | 1 | 1 | 2 | 2 | 0.36047 | A | 2A2 |
| 39 | 1 | 1 | 3 | 1 | 0.38105 | A | 1A3 |
| 40 | 1 | 2 | 2 | 6 | 0.39888 | B | 6B2 |
| 41 | 1 | 1 | 2 | 1 | 0.40441 | A | 1A2 |
| 42 | 1 | 2 | 1 | 6 | 0.42695 | B | 6B1 |
| 43 | 1 | 3 | 1 | 8 | 0.43967 | C | 8C1 |
| 44 | 1 | 1 | 2 | 6 | 0.44954 | A | 6A2 |
| 45 | 1 | 3 | 2 | 6 | 0.47464 | C | 6C2 |
| 46 | 1 | 1 | 1 | 8 | 0.48023 | A | 8A1 |
| 47 | 1 | 1 | 1 | 7 | 0.48398 | A | 7A1 |

Distribution
began at 1345
terminated at 1500
JW
5SEPT2012

File Folder: 11a

Item Number: 2

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Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical subs 2
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Shawano whole water column tank treatment

AEH-12-PSEUDO-04

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 48 | 1 | 2 | 3 | 8 | 0.48900 | B | 8B3 |
| 49 | 1 | 2 | 2 | 2 | 0.50511 | B | 2B2 |
| 50 | 1 | 2 | 2 | 8 | 0.52181 | B | 8B2 |
| 51 | 1 | 1 | 3 | 9 | 0.52488 | A | 9A3 |
| 52 | 1 | 2 | 3 | 6 | 0.53810 | B | 6B3 |
| 53 | 1 | 2 | 2 | 9 | 0.54061 | B | 9B2 |
| 54 | 1 | 2 | 1 | 4 | 0.56338 | B | 4B1 |
| 55 | 1 | 3 | 3 | 7 | 0.60611 | C | 7C3 |
| 56 | 1 | 3 | 1 | 3 | 0.60927 | C | 3C1 |
| 57 | 1 | 1 | 2 | 4 | 0.62026 | A | 4A2 |
| 58 | 1 | 1 | 3 | 5 | 0.63012 | A | 5A3 |
| 59 | 1 | 1 | 1 | 6 | 0.65625 | A | 6A1 |
| 60 | 1 | 3 | 2 | 9 | 0.66940 | C | 9C2 |
| 61 | 1 | 3 | 1 | 4 | 0.68710 | C | 4C1 |
| 62 | 1 | 3 | 1 | 5 | 0.69054 | C | 5C1 |
| 63 | 1 | 1 | 3 | 6 | 0.71055 | A | 6A3 |
| 64 | 1 | 3 | 2 | 1 | 0.73661 | C | 1C2 |
| 65 | 1 | 2 | 3 | 1 | 0.75311 | B | 1B3 |
| 66 | 1 | 3 | 1 | 6 | 0.76155 | C | 6C1 |
| 67 | 1 | 1 | 3 | 8 | 0.76411 | A | 8A3 |
| 68 | 1 | 2 | 1 | 5 | 0.76527 | B | 5B1 |
| 69 | 1 | 2 | 1 | 2 | 0.76674 | B | 2B1 |
| 70 | 1 | 3 | 3 | 8 | 0.77423 | C | 8C3 |
| 71 | 1 | 3 | 3 | 3 | 0.79781 | C | 3C3 |
| 72 | 1 | 2 | 3 | 7 | 0.81154 | B | 7B3 |
| 73 | 1 | 2 | 1 | 9 | 0.82249 | B | 9B1 |
| 74 | 1 | 1 | 2 | 9 | 0.83782 | A | 9A2 |
| 75 | 1 | 1 | 1 | 3 | 0.85976 | A | 3A1 |
| 76 | 1 | 3 | 2 | 5 | 0.86941 | C | 5C2 |
| 77 | 1 | 1 | 1 | 5 | 0.89111 | A | 5A1 |
| 78 | 1 | 2 | 1 | 1 | 0.89793 | B | 1B1 |
| 79 | 1 | 1 | 1 | 9 | 0.90048 | A | 9A1 |
| 80 | 1 | 1 | 1 | 1 | 0.91950 | A | 1A1 |
| 81 | 1 | 3 | 2 | 2 | 0.98447 | C | 2C2 |

File Folder: ______

Item Number: ______

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Page ______ of ______
analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12

```
/*********************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : AUGUST 11, 2012 - JAL
*  Verified by:  _______ (Date:_______)                    page ____ of ____
*  Random allocation of trays to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2, or 3, e
   round = distribution round, place one tray in the assigned position (9 per test replicate - 3 for

/************************************************************************************************

/*Location and exposure type: Shawano - whole water column treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
 if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
  if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
```

AEH-12-PSEUDO-04

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```
if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
if row = 1 and tank = 4 and position = 3 then tankn =  4A3';
if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
if row = 2 and tank = 7 and position = 2 then tankn =  7B2';
if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
if row = 1 and tank = 9 and position = 1 tnen tankn = '9A1';
if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
```

~~AEH-12-P~~
WL 29APR2014

AEH-12-PSEUDO-04

```
        if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
         if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
          if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
           if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
            if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;
proc sort data= glochidiadist;
 by round x;
 run;
proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artif
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Shawano whole water column tank treatment';
run;
```

AEH-12-PSEUDO-04

8/11/12
JA

Page 5 of 8

```
 *  date created   : AUGUST 11, 2012 - JAL JP
 *  Verified by:  _______ (Date:_______)                    page ____ of ____
 *  Random allocation of trays to tank.sas
 *******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;                 AEM-12-PSEUDO-04

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2,
or 3, etc)
   round = distribution round, place one tray in the assigned position (9 per test replicate -
 3 for each exposure duration) */

/*******************************************************************************************
********/

/*Location and exposure type: Shawano - whole water column treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 81 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A ;
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';   Page 6 of 8
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
```

```
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
 if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
  if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
   if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
    if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
     if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
      if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
       if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
        if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
 if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
  if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
   if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
    if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
     if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
      if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
       if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
        if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
 if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
  if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
   if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
    if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
     if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
      if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
       if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
        if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
 if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
  if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
   if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
    if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
     if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
      if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
       if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
        if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
 if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
  if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
   if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
    if row = 2 and tank = 7 and position = 2 then tankn = '7B2';
     if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
      if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
       if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
        if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
 if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
  if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
   if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
    if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
```

AEH-12-PSEUDO-04

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```
      if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
       if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
        if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
         if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
 if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
  if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
   if row = 2 and tank = 9 and position = 1 then tankn = '9B1 ;
    if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
     if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
      if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
       if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
        if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.07 seconds
      cpu time            0.07 seconds


proc sort data= glochidiadist;
 by round x;
 run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels
on artifical substrates';
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Shawano whole water column tank treatment';
run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-12-PSEUDO-04

8/11/12

FF # 11a
Item No. 2
Pg 6 of 8

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra musses1 on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 1 ***
Shawano - whole water body Treatment

8/13/12
JS

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.03194 | 1A1 | 6h |
| 2 | 2 | 1 | 0.03514 | 1B1 | 6h |
| 3 | 2 | 2 | 0.28180 | 1B2 | 6h |
| 4 | 3 | 1 | 0.49047 | 1C1 | 9h |
| 5 | 1 | 3 | 0.70588 | 1A3 | 9h |
| 6 | 2 | 3 | 0.72184 | 1B3 | 9h |
| 7 | 3 | 3 | 0.75338 | 1C3 | 12 |
| 8 | 3 | 2 | 0.80186 | 1C2 | 12 |
| 9 | 1 | 2 | 0.95124 | 1A2 | 12 |

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Item Number: 3

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 2 ***
Shawano - whole water body Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.19283 | 2A1 | 6h |
| 2 | 2 | 1 | 0.22382 | 2B1 | 6h |
| 3 | 3 | 1 | 0.24148 | 2C1 | 6h |
| 4 | 2 | 2 | 0.28394 | 2B2 | 9h |
| 5 | 1 | 3 | 0.29763 | 2A3 | 9h |
| 6 | 3 | 3 | 0.62146 | 2C3 | 9h |
| 7 | 3 | 2 | 0.71062 | 2C2 | 12 |
| 8 | 1 | 2 | 0.79088 | 2A2 | 12 |
| 9 | 2 | 3 | 0.98840 | 2B3 | 12 |

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 3 ***
Shawano - whole water body Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 0.25496 | 3B3 | 6h |
| 2 | 2 | 2 | 0.28609 | 3B2 | 6h |
| 3 | 1 | 1 | 0.35372 | 3A1 | 6h |
| 4 | 2 | 1 | 0.41249 | 3B1 | 9h |
| 5 | 3 | 3 | 0.48955 | 3C3 | 9h |
| 6 | 3 | 2 | 0.61938 | 3C2 | 9h |
| 7 | 1 | 2 | 0.63052 | 3A2 | 12 |
| 8 | 1 | 3 | 0.88938 | 3A3 | 12 |
| 9 | 3 | 1 | 0.99249 | 3C1 | 12 |

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 4 ***
Shawano - whole water body Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.23640 | 4A1 | 6h |
| 2 | 2 | 1 | 0.26349 | 4B1 | 6h |
| 3 | 1 | 2 | 0.36924 | 4A2 | 6h |
| 4 | 1 | 3 | 0.56305 | 4A3 | 9h |
| 5 | 2 | 2 | 0.56965 | 4B2 | 9h |
| 6 | 3 | 1 | 0.70154 | 4C1 | 9h |
| 7 | 3 | 2 | 0.70889 | 4C2 | 12 |
| 8 | 2 | 3 | 0.76343 | 4B3 | 12 |
| 9 | 3 | 3 | 0.83244 | 4C3 | 12 |

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Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 5 ***
Shawano - whole water body Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.19283 | 2A1 | 6h |
| 2 | 2 | 1 | 0.22382 | 2B1 | 6h |
| 3 | 3 | 1 | 0.24148 | 2C1 | 6h |
| 4 | 2 | 2 | 0.28394 | 2B2 | 9h |
| 5 | 1 | 3 | 0.29763 | 2A3 | 9h |
| 6 | 3 | 3 | 0.62146 | 2C3 | 9h |
| 7 | 3 | 2 | 0.71062 | 2C2 | 12 |
| 8 | 1 | 2 | 0.79088 | 2A2 | 12 |
| 9 | 2 | 3 | 0.98840 | 2B3 | 12 |

(1) Tank numbers should be 5 not 2. KW 6SEPT12

See Deviation #2 for further clarification. KW 19NOV13

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Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 6 ***
Shawano - whole water body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 0.14871 | 6A2 | 6h |
| 2 | 1 | 3 | 0.45067 | 6A3 | 6h |
| 3 | 1 | 1 | 0.47774 | 6A1 | 6h |
| 4 | 2 | 1 | 0.54650 | 6B1 | 9h |
| 5 | 3 | 2 | 0.57203 | 6C2 | 9h |
| 6 | 2 | 2 | 0.57288 | 6B2 | 9h |
| 7 | 3 | 3 | 0.63457 | 6C3 | 12 |
| 8 | 2 | 3 | 0.66328 | 6B3 | 12 |
| 9 | 3 | 1 | 0.82805 | 6C1 | 12 |

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 7 ***
Shawano - whole water body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.19283 | ① 2A1 | 6h |
| 2 | 2 | 1 | 0.22382 | 2B1 | 6h |
| 3 | 3 | 1 | 0.24148 | 2C1 | 6h |
| 4 | 2 | 2 | 0.28394 | 2B2 | 9h |
| 5 | 1 | 3 | 0.29763 | 2A3 | 9h |
| 6 | 3 | 3 | 0.62146 | 2C3 | 9h |
| 7 | 3 | 2 | 0.71062 | 2C2 | 12 |
| 8 | 1 | 2 | 0.79088 | 2A2 | 12 |
| 9 | 2 | 3 | 0.98840 | 2B3 | 12 |

① Tank numbers should be 7 not 2. KW 6SEPT12

See Deviation #2 for further clarification. KW 19NOV13

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 8 ***
Shawano - whole water body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 0.12552 | 8B3 | 6h |
| 2 | 1 | 3 | 0.14940 | 8A3 | 6h |
| 3 | 3 | 1 | 0.28780 | 8C1 | 6h |
| 4 | 1 | 1 | 0.31796 | 8A1 | 9h |
| 5 | 3 | 2 | 0.36760 | 8C2 | 9h |
| 6 | 2 | 1 | 0.45120 | 8B1 | 9h |
| 7 | 1 | 2 | 0.48872 | 8A2 | 12 |
| 8 | 3 | 3 | 0.73228 | 8C3 | 12 |
| 9 | 2 | 2 | 0.93485 | 8B2 | 12 |

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 JTL

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 9 ***
Shawano - whole water body Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.19283 | (1) 2A1 | 6h |
| 2 | 2 | 1 | 0.22382 | 2B1 | 6h |
| 3 | 3 | 1 | 0.24148 | 2C1 | 6h |
| 4 | 2 | 2 | 0.28394 | 2B2 | 9h |
| 5 | 1 | 3 | 0.29763 | 2A3 | 9h |
| 6 | 3 | 3 | 0.62146 | 2C3 | 9h |
| 7 | 3 | 2 | 0.71062 | 2C2 | 12 |
| 8 | 1 | 2 | 0.79088 | 2A2 | 12 |
| 9 | 2 | 3 | 0.98840 | 2B3 | 12 |

(1) Tank numbers should be 9 not 2. KW 6 SEPT12

See Deviation #2 for further clarification

A[illegible]-12-PSEUDO-04

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

```
/*******************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 13 August 2012 - JAL
*  Verified by:   _______ (Date:_______)                page ____ of ____
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=105 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
                          Substrate removal from tanks. See title below.
/*Random assignment of ~~treatment to experimental tanks~~*/
/*Location/exposure type: Shawano - whole tank treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
     run;
proc sort data=TANK1A;
 by x;
run;

data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
```

AEH-12-PSEUDO-04

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AEH-12-PSEUDO-04

```
data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
    if row = 2 and position = 1 then tankn = '2B1';
     if row = 2 and position = 2 then tankn = '2B2';
      if row = 2 and position = 3 then tankn = '2B3';
       if row = 3 and position = 1 then tankn = '2C1';
        if row = 3 and position = 2 then tankn = '2C2';
         if row = 3 and position = 3 then tankn = '2C3';
     run;
proc sort data=TANK2A;
 by x;
run;

data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
```

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```
       if row = 3 and position = 1 then tankn = '3C1';
        if row = 3 and position = 2 then tankn = '3C2';
         if row = 3 and position = 3 then tankn = '3C3';
     run;
proc sort data=TANK3A;
 by x;
run;

data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _r_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;
proc sort data=TANK4A;
 by x;
run;

data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
```

```
    if _n_ = 8 then trt = '12h ;
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK4A;
title1 h=2  Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK5A; set TANK5;
 if row = 1 and position = 1 then tankn = '5A1';
  if row = 1 and position = 2 then tankn = '5A2';
   if row = 1 and position = 3 then tankn = '5A3';
    if row = 2 and position = 1 then tankn = '5B1';
     if row = 2 and position = 2 then tankn = '5B2';
      if row = 2 and position = 3 then tankn = '5B3';
       if row = 3 and position = 1 then tankn = '5C1';
        if row = 3 and position = 2 then tankn = '5C2';
         if row = 3 and position = 3 then tankn = '5C3';
     run;
proc sort data=TANK5A;
 by x;
run;

data assign_trt_TANK5A; set TANK5A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  ond;
 end;
```

```
run;
data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;
proc sort data=TANK6A;
 by x;
run;

data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn =  7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
    run;
proc sort data=TANK7A;
 by x;
run;
```

AEH-12-PSEUDO-04

```
data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;
proc sort data=TANK8A;
 by x;
run;

data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04 ;
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Shawano - whole water body Treatment ';
```

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AEH-12-PSEUDO-04

```
run;
data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn =  9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
     run;
proc sort data=TANK9A;
 by x;
run;

data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussosl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Shawano - whole water body Treatnent ';
run;
```

8/13/12

J.n

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```
*  date created  : 13 August 2012 - JAL 8/13/12
*  Verified by:  ______ (Date:______)                          page ____ of ____
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=105 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
                         Substrate removal from tanks. See title on next page. 28APR14
/*Random assignment of ~~treatment to experimental tanks*~~/
/*Location/exposure type: Shawano - whole tank treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK1 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK1.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK1A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

```
data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.ASSIGN_TRT_TANK1A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK1A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK2 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds

data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
```

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```
    if row = 2 and position = 1 then tankn = '2B1';
     if row = 2 and position = 2 then tankn = '2B2';
      if row = 2 and position = 3 then tankn = '2B3';
       if row = 3 and position = 1 then tankn = '2C1';
        if row = 3 and position = 2 then tankn = '2C2';
         if row = 3 and position = 3 then tankn = '2C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK2.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK2A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt =  9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.ASSIGN_TRT_TANK2A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;
```

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```
NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
```

```
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK3 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
       if row = 3 and position = 1 then tankn = '3C1';
        if row = 3 and position = 2 then tankn = '3C2';
         if row = 3 and position = 3 then tankn = '3C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK3.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK3A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
```

```
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h ;
  run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.ASSIGN_TRT_TANK3A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK3A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK4 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK4.
```

```
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK4A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.ASSIGN_TRT_TANK4A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK4A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
```

```
   end;
  end;
 run;

NOTE: The data set WORK.TANK5 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


 data TANK5A; set TANK5;
  if row = 1 and position = 1 then tankn = '5A1';
   if row = 1 and position = 2 then tankn = '5A2';
    if row = 1 and position = 3 then tankn = '5A3';
     if row = 2 and position = 1 then tankn = '5B1';
      if row = 2 and position = 2 then tankn = '5B2';
       if row = 2 and position = 3 then tankn = '5B3';
        if row = 3 and position = 1 then tankn = '5C1';
         if row = 3 and position = 2 then tankn = '5C2';
          if row = 3 and position = 3 then tankn = '5C3';
      run;

NOTE: There were 9 observations read from the data set WORK.TANK5.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


 proc sort data=TANK5A;
  by x;
 run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



 data assign_trt_TANK5A; set TANK5A;
  if _n_ = 1 then trt = '6h';
   if _n_ = 2 then trt = '6h';
    if _n_ = 3 then trt = '6h';
     if _n_ = 4 then trt = '9h';
  if _n_ = 5 then trt = '9h';
   if _n_ = 6 then trt = '9h';
    if _n_ = 7 then trt = '12h';
     if _n_ = 8 then trt = '12h';
  if _n_ = 9 then trt = '12h';
   run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.ASSIGN_TRT_TANK5A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
```

```
      real time           0.01 seconds
      cpu time            0.01 seconds
```



```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK6 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK6.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=TANK6A;
 by x;
run;
```

```
NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.ASSIGN_TRT_TANK6A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK6A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK7 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-12-PSEUDO-04

```
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn = '7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK7.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK7A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.ASSIGN_TRT_TANK7A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds
```

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```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Shawano - whole water body Treatment ';
```

```
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK8 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK8.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK8A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```



```

data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
```

```
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.ASSIGN_TRT_TANK8A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK8A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK9 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
```

```
run;

NOTE: There were 9 observations read from the data set WORK.TANK9.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=TANK9A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.ASSIGN_TRT_TANK9A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
! artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Shawano - whole water body Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

8/13/12 JA

FF # 11a
Item No. 3
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```
NOTE: This SAS session is using a registry in WORK.  All changes will be lost at the end of this sess
```

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrat 1
AEH-12-PSEUDO-04
Random assignment of treatment to experimental tanks
Treatment Location/type: Shawano - bottom injection

8/11/12
JA

AEH-12-PSEUDO-04

| Obs | block | tank | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 9 | 0.13021 | Tank 9 | control |
| 2 | 1 | 2 | 0.15287 | Tank 2 | 50 |
| 3 | 1 | 4 | 0.23351 | Tank 4 | 100 |
| 4 | 1 | 1 | 0.26174 | Tank 1 | control |
| 5 | 1 | 7 | 0.29656 | Tank 7 | 50 |
| 6 | 1 | 5 | 0.52465 | Tank 5 | 100 |
| 7 | 1 | 8 | 0.67430 | Tank 8 | control |
| 8 | 1 | 3 | 0.91450 | Tank 3 | 50 |
| 9 | 1 | 6 | 0.99600 | Tank 6 | 100 |

File Folder: 14a Item Number: 1 Page 1 of 4

Analysis performed by J. Luoma SAS version 9.2 08:59 11AUG12

```
/*******************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 11 August 2012 - JAL JAS 8/11/12
*  Verified by:  _______ (Date:_______)                page ____ of ____    AEH-12-PSEUDO-04
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Shawano - bottom injection exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;
data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn = 'Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;
proc sort data=fish2;
 by block x;
run;

data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;
proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Shawano - bottom injection';
run;
```

Page 2 of 4

```
* date created  : 11 August 2012 - JAL
* Verified by:  ______ (Date:______)                    page ____ of ____
* Random allocation of treatment to tank.sas
.***********************************************************************/   AEH-12-PSEUDO-04
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;'
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random assignment of treatment to experimental tanks*/
/*Location/exposure type: Shawano - bottom injection exposure*/
data fish;
 do block = 1 to 1 by 1;
  do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
 end;
run;

NOTE: The data set WORK.FISH has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data fish2; set fish;
 if block = 1 and tank = 1 then tankn = 'Tank 1';
  if block = 1 and tank = 2 then tankn = 'Tank 2';
   if block = 1 and tank = 3 then tankn = 'Tank 3';
    if block = 1 and tank = 4 then tankn = 'Tank 4';
     if block = 1 and tank = 5 then tankn = 'Tank 5';
      if block = 1 and tank = 6 then tankn =  Tank 6';
       if block = 1 and tank = 7 then tankn = 'Tank 7';
        if block = 1 and tank = 8 then tankn = 'Tank 8';
         if block = 1 and tank = 9 then tankn = 'Tank 9';
     run;

NOTE: There were 9 observations read from the data set WORK.FISH.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=fish2;
 by block x;
run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.FISH2 has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

FF # 14a
Item No. 1
Pg 3 of 4

AEH-12-PSEUDO-04

```
data assign_trt_fish; set fish2;
 if _n_ = 1 then trt = 'control';
  if _n_ = 2 then trt = '50';
   if _n_ = 3 then trt = '100';
    if _n_ = 4 then trt = 'control';
 if _n_ = 5 then trt = '50';
  if _n_ = 6 then trt = '100';
   if _n_ = 7 then trt = 'control';
    if _n_ = 8 then trt = '50';
 if _n_ = 9 then trt = '100';
  run;

NOTE: There were 9 observations read from the data set WORK.FISH2.
NOTE: The data set WORK.ASSIGN_TRT_FISH has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.06 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_fish;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of treatment to experimental tanks';
title4 h=1 'Treatment Location/type: Shawano - bottom injection';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_FISH.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

8/1/12
JN

FF # 14a
Item No. 1
Pg 4 of 4

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical subs 1
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Shawano bottom injection tank treatment

8/11/12
Jrn

**AEH-12-PSEUDO-04**

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 2 | 8 | 0.00810 | A | 8A2 |
| 2 | 1 | 3 | 3 | 2 | 0.03556 | C | 2C3 |
| 3 | 1 | 1 | 2 | 6 | 0.03743 | A | 6A2 |
| 4 | 1 | 2 | 1 | 5 | 0.03887 | B | 5B1 |
| 5 | 1 | 1 | 1 | 2 | 0.06626 | A | 2A1 |
| 6 | 1 | 2 | 1 | 2 | 0.06878 | B | 2B1 |
| 7 | 1 | 1 | 1 | 1 | 0.08357 | A | 1A1 |
| 8 | 1 | 1 | 2 | 1 | 0.09108 | A | 1A2 |
| 9 | 1 | 2 | 2 | 9 | 0.12185 | B | 9B2 |
| 10 | 1 | 2 | 3 | 1 | 0.12192 | B | 1B3 |
| 11 | 1 | 3 | 1 | 4 | 0.14635 | C | 4C1 |
| 12 | 1 | 1 | 2 | 2 | 0.17909 | A | 2A2 |
| 13 | 1 | 1 | 3 | 6 | 0.19369 | A | 6A3 |
| 14 | 1 | 1 | 3 | 4 | 0.19825 | A | 4A3 |
| 15 | 1 | 2 | 2 | 6 | 0.20476 | B | 6B2 |
| 16 | 1 | 2 | 3 | 8 | 0.21165 | B | 8B3 |
| 17 | 1 | 3 | 1 | 6 | 0.22199 | C | 6C1 |
| 18 | 1 | 3 | 3 | 5 | 0.22663 | C | 5C3 |
| 19 | 1 | 3 | 3 | 1 | 0.23721 | C | 1C3 |
| 20 | 1 | 2 | 3 | 4 | 0.23995 | B | 4B3 |
| 21 | 1 | 2 | 2 | 1 | 0.25274 | B | 1B2 |
| 22 | 1 | 1 | 3 | 7 | 0.25387 | A | 7A3 |
| 23 | 1 | 1 | 3 | 5 | 0.27644 | A | 5A3 |
| 24 | 1 | 3 | 2 | 2 | 0.27738 | C | 2C2 |
| 25 | 1 | 3 | 2 | 4 | 0.28281 | C | 4C2 |
| 26 | 1 | 1 | 3 | 8 | 0.28295 | A | 8A3 |
| 27 | 1 | 3 | 2 | 5 | 0.29153 | C | 5C2 |
| 28 | 1 | 3 | 1 | 2 | 0.30627 | C | 2C1 |
| 29 | 1 | 1 | 2 | 5 | 0.32998 | A | 5A2 |
| 30 | 1 | 2 | 1 | 1 | 0.33341 | B | 1B1 |
| 31 | 1 | 1 | 3 | 1 | 0.34612 | A | 1A3 |
| 32 | 1 | 2 | 3 | 7 | 0.36184 | B | 7B3 |
| 33 | 1 | 2 | 3 | 9 | 0.43563 | B | 9B3 |
| 34 | 1 | 3 | 3 | 7 | 0.44991 | C | 7C3 |
| 35 | 1 | 1 | 1 | 5 | 0.47095 | A | 5A1 |
| 36 | 1 | 1 | 3 | 2 | 0.47688 | A | 2A3 |
| 37 | 1 | 2 | 3 | 3 | 0.47977 | B | 3B3 |
| 38 | 1 | 3 | 3 | 4 | 0.55220 | C | 4C3 |
| 39 | 1 | 2 | 2 | 7 | 0.55570 | B | 7B2 |
| 40 | 1 | 1 | 1 | 6 | 0.56422 | A | 6A1 |
| 41 | 1 | 3 | 3 | 3 | 0.56430 | C | 3C3 |
| 42 | 1 | 2 | 1 | 9 | 0.56500 | B | 9B1 |
| 43 | 1 | 3 | 2 | 8 | 0.57148 | C | 8C2 |
| 44 | 1 | 1 | 2 | 4 | 0.60142 | A | 4A2 |
| 45 | 1 | 2 | 2 | 8 | 0.60482 | B | 8B2 |
| 46 | 1 | 1 | 3 | 3 | 0.60611 | A | 3A3 |
| 47 | 1 | 2 | 3 | 5 | 0.62329 | B | 5B3 |

✓ indicates bag positions to be used for 12 h treatment.
KW
7SEPT12

Distribution began at 1000.

Distribution complete at 1033. KW
7SEP12

**File Folder:** 149

**Item Number:** 2

**Page** 1 **of** 8

Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12

Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artifical subs 2
AEH-12-PSUEDO-04
Random assignment of trays to test tank/position
Test Location/type = Shawano bottom injection tank treatment

AEH-12-PSEUDO-04
JWL 29APR2014

AEH-12-PSEUDO-04

| Obs | round | row | position | tank | x | _row_ | tankn |
|---|---|---|---|---|---|---|---|
| 48 | 1 | 1 | 1 | 7 | 0.65077 | A | 7A1 |
| 49 | 1 | 1 | 1 | 4 | 0.67036 | A | 4A1 |
| 50 | 1 | 3 | 3 | 9 | 0.67289 | C | 9C3 |
| 51 | 1 | 2 | 1 | 6 | 0.68660 | B | 6B1 |
| 52 | 1 | 2 | 2 | 3 | 0.70985 | B | 3B2 |
| 53 | 1 | 1 | 2 | 3 | 0.71071 | A | 3A2 |
| 54 | 1 | 3 | 1 | 5 | 0.74940 | C | 5C1 |
| 55 | 1 | 2 | 3 | 6 | 0.75203 | B | 6B3 |
| 56 | 1 | 3 | 1 | 9 | 0.75647 | C | 9C1 |
| 57 | 1 | 1 | 1 | 3 | 0.76245 | A | 3A1 |
| 58 | 1 | 2 | 2 | 4 | 0.76310 | B | 4B2 |
| 59 | 1 | 2 | 3 | 2 | 0.76913 | B | 2B3 |
| 60 | 1 | 1 | 1 | 9 | 0.77002 | A | 9A1 |
| 61 | 1 | 2 | 2 | 2 | 0.77972 | B | 2B2 |
| 62 | 1 | 1 | 3 | 9 | 0.81719 | A | 9A3 |
| 63 | 1 | 3 | 1 | 7 | 0.82828 | C | 7C1 |
| 64 | 1 | 1 | 2 | 9 | 0.83206 | A | 9A2 |
| 65 | 1 | 3 | 1 | 1 | 0.83905 | C | 1C1 |
| 66 | 1 | 3 | 3 | 8 | 0.86796 | C | 8C3 |
| 67 | 1 | 3 | 2 | 1 | 0.88677 | C | 1C2 |
| 68 | 1 | 2 | 2 | 5 | 0.89956 | B | 5B2 |
| 69 | 1 | 1 | 1 | 8 | 0.90778 | A | 8A1 |
| 70 | 1 | 3 | 1 | 3 | 0.91931 | C | 3C1 |
| 71 | 1 | 2 | 1 | 4 | 0.92470 | B | 4B1 |
| 72 | 1 | 2 | 1 | 8 | 0.92861 | B | 8B1 |
| 73 | 1 | 3 | 2 | 3 | 0.94083 | C | 3C2 |
| 74 | 1 | 2 | 1 | 3 | 0.94313 | B | 3B1 |
| 75 | 1 | 3 | 1 | 8 | 0.95355 | C | 8C1 |
| 76 | 1 | 2 | 1 | 7 | 0.95623 | B | 7B1 |
| 77 | 1 | 3 | 2 | 9 | 0.97167 | C | 9C2 |
| 78 | 1 | 3 | 3 | 6 | 0.97857 | C | 6C3 |
| 79 | 1 | 3 | 2 | 6 | 0.98284 | C | 6C2 |
| 80 | 1 | 3 | 2 | 7 | 0.99054 | C | 7C2 |
| 81 | 1 | 1 | 2 | 7 | 0.99696 | A | 7A2 |

Page 2 of 8

Analysis performed by J. Luoma SAS version 9.2 10:48 11AUG12 JL

```
/*******************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : AUGUST 11, 2012 - JAL  JAL
*  Verified by:  _______ (Date:_____)                page ____ of ____   AEH-12-PSEUDO-04
*  Random allocation of trays to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2, or 3, e
   round = distribution round, place one tray in the assigned position (9 per test replicate - 3 for

/*************************************************************************************************

/*Location and exposure type: Shawano - bottom injection treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;
data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 anc position = 3 tnen tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
 if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
  if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
```

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```
if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
if row = 3 and tank = 4 and position = 3 then tankn = '4C3 ;
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
if row = 2 and tank = 7 and position = 2 then tankn =  7B2';
if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
```

```
     if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
      if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
       if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
        if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
         if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;
proc sort data= glochidiadist;
 by round x;
 run;
proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels on artif
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Shawano bottom injection tank treatment';
run;
```

AEH-12-PSEUDO-04

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AEH-12-PSEUDO-04

```
*  date created  : AUGUST 11, 2012 - JAL Jn
*  Verified by:  _______ (Date:______)                         page ____ of ____
*  Random allocation of trays to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options /*ls=85 ps=40 formdlim='-' */ pageno = 1 nocenter nodate nosource2;

/*Random distribution of trays to experimental tanks*/
/* tanks 1 to 9 = tank 1 row A,B,C, each row has 3 positions (ie: Tank 1 row A position 1, 2,
or 3, etc)
   round = distribution round, place one tray in the assigned position (9 per test replicate -
 3 for each exposure duration) */

/*****************************************************************************************
********/

/*Location and exposure type: Shawano - bottom injection treatment*/
data glochidia;
 do round = 1 to 1 by 1;
 do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
 do tank = 1 to 9 by 1;
   x = ranuni(-1);
   output;
  end;
  end;
 end;
 end;
run;

NOTE: The data set WORK.GLOCHIDIA has 81 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


data glochidiadist; set glochidia;
if row = 1 then _row_ = 'A';
if row = 2 then _row_ = 'B';
if row = 3 then _row_ = 'C';
 if row = 1 and tank = 1 and position = 1 then tankn = '1A1';
  if row = 1 and tank = 1 and position = 2 then tankn = '1A2';
   if row = 1 and tank = 1 and position = 3 then tankn = '1A3';
    if row = 2 and tank = 1 and position = 1 then tankn = '1B1';
     if row = 2 and tank = 1 and position = 2 then tankn = '1B2';
      if row = 2 and tank = 1 and position = 3 then tankn = '1B3';
       if row = 3 and tank = 1 and position = 1 then tankn = '1C1';
        if row = 3 and tank = 1 and position = 2 then tankn = '1C2';
         if row = 3 and tank = 1 and position = 3 then tankn = '1C3';
 if row = 1 and tank = 2 and position = 1 then tankn = '2A1';
  if row = 1 and tank = 2 and position = 2 then tankn = '2A2';
   if row = 1 and tank = 2 and position = 3 then tankn = '2A3';
```

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```
    if row = 2 and tank = 2 and position = 1 then tankn = '2B1';
     if row = 2 and tank = 2 and position = 2 then tankn = '2B2';
      if row = 2 and tank = 2 and position = 3 then tankn = '2B3';
       if row = 3 and tank = 2 and position = 1 then tankn = '2C1';
        if row = 3 and tank = 2 and position = 2 then tankn = '2C2';
         if row = 3 and tank = 2 and position = 3 then tankn = '2C3';
if row = 1 and tank = 3 and position = 1 then tankn = '3A1';
 if row = 1 and tank = 3 and position = 2 then tankn = '3A2';
  if row = 1 and tank = 3 and position = 3 then tankn = '3A3';
   if row = 2 and tank = 3 and position = 1 then tankn = '3B1';
    if row = 2 and tank = 3 and position = 2 then tankn = '3B2';
     if row = 2 and tank = 3 and position = 3 then tankn = '3B3';
      if row = 3 and tank = 3 and position = 1 then tankn = '3C1';
       if row = 3 and tank = 3 and position = 2 then tankn = '3C2';
        if row = 3 and tank = 3 and position = 3 then tankn = '3C3';
if row = 1 and tank = 4 and position = 1 then tankn = '4A1';
 if row = 1 and tank = 4 and position = 2 then tankn = '4A2';
  if row = 1 and tank = 4 and position = 3 then tankn = '4A3';
   if row = 2 and tank = 4 and position = 1 then tankn = '4B1';
    if row = 2 and tank = 4 and position = 2 then tankn = '4B2';
     if row = 2 and tank = 4 and position = 3 then tankn = '4B3';
      if row = 3 and tank = 4 and position = 1 then tankn = '4C1';
       if row = 3 and tank = 4 and position = 2 then tankn = '4C2';
        if row = 3 and tank = 4 and position = 3 then tankn = '4C3';
if row = 1 and tank = 5 and position = 1 then tankn = '5A1';
 if row = 1 and tank = 5 and position = 2 then tankn = '5A2';
  if row = 1 and tank = 5 and position = 3 then tankn = '5A3';
   if row = 2 and tank = 5 and position = 1 then tankn = '5B1';
    if row = 2 and tank = 5 and position = 2 then tankn = '5B2';
     if row = 2 and tank = 5 and position = 3 then tankn = '5B3';
      if row = 3 and tank = 5 and position = 1 then tankn = '5C1';
       if row = 3 and tank = 5 and position = 2 then tankn = '5C2';
        if row = 3 and tank = 5 and position = 3 then tankn = '5C3';
if row = 1 and tank = 6 and position = 1 then tankn = '6A1';
 if row = 1 and tank = 6 and position = 2 then tankn = '6A2';
  if row = 1 and tank = 6 and position = 3 then tankn = '6A3';
   if row = 2 and tank = 6 and position = 1 then tankn = '6B1';
    if row = 2 and tank = 6 and position = 2 then tankn = '6B2';
     if row = 2 and tank = 6 and position = 3 then tankn = '6B3';
      if row = 3 and tank = 6 and position = 1 then tankn = '6C1';
       if row = 3 and tank = 6 and position = 2 then tankn = '6C2';
        if row = 3 and tank = 6 and position = 3 then tankn = '6C3';
if row = 1 and tank = 7 and position = 1 then tankn = '7A1';
 if row = 1 and tank = 7 and position = 2 then tankn = '7A2';
  if row = 1 and tank = 7 and position = 3 then tankn = '7A3';
   if row = 2 and tank = 7 and position = 1 then tankn = '7B1';
    if row = 2 and tank = 7 and position = 2 then tankn = '7B2';
     if row = 2 and tank = 7 and position = 3 then tankn = '7B3';
      if row = 3 and tank = 7 and position = 1 then tankn = '7C1';
       if row = 3 and tank = 7 and position = 2 then tankn = '7C2';
        if row = 3 and tank = 7 and position = 3 then tankn = '7C3';
if row = 1 and tank = 8 and position = 1 then tankn = '8A1';
 if row = 1 and tank = 8 and position = 2 then tankn = '8A2';
  if row = 1 and tank = 8 and position = 3 then tankn = '8A3';
   if row = 2 and tank = 8 and position = 1 then tankn = '8B1';
    if row = 2 and tank = 8 and position = 2 then tankn = '8B2';
```

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```
      if row = 2 and tank = 8 and position = 3 then tankn = '8B3';
       if row = 3 and tank = 8 and position = 1 then tankn = '8C1';
        if row = 3 and tank = 8 and position = 2 then tankn = '8C2';
         if row = 3 and tank = 8 and position = 3 then tankn = '8C3';
 if row = 1 and tank = 9 and position = 1 then tankn = '9A1';
  if row = 1 and tank = 9 and position = 2 then tankn = '9A2';
   if row = 1 and tank = 9 and position = 3 then tankn = '9A3';
    if row = 2 and tank = 9 and position = 1 then tankn = '9B1';
     if row = 2 and tank = 9 and position = 2 then tankn = '9B2';
      if row = 2 and tank = 9 and position = 3 then tankn = '9B3';
       if row = 3 and tank = 9 and position = 1 then tankn = '9C1';
        if row = 3 and tank = 9 and position = 2 then tankn = '9C2';
         if row = 3 and tank = 9 and position = 3 then tankn = '9C3';
Run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIA.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.07 seconds
      cpu time            0.07 seconds


proc sort data= glochidiadist;
 by round x;
 run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: The data set WORK.GLOCHIDIADIST has 81 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data = glochidiadist;
title1 h=2 'Efficacy of Psuedomonas fluorescens (Pf-CL145A)SDP for controlling zebra mussels
on artifical substrates';
title2 h=1.5 'AEH-12-PSUEDO-04';
title3 h=1 'Random assignment of trays to test tank/position';
title4 h=1 'Test Location/type = Shawano bottom injection tank treatment';
run;

NOTE: There were 81 observations read from the data set WORK.GLOCHIDIADIST.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

AEH-12-PSEUDO-04

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JAL

FF # 14a
Item No. 2
Pg 8 of 8

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 1 ***
Shawano - bottom injection Treatment

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SA

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | ~~1~~ | 1 | 0.00558 | 1A1 | 6h |
| 2 | 2 | ~~1~~ | 0.07369 | 1B1 | 6h |
| 3 | 2 | 3 | ~~0.10023~~ | 1B3 | 6h |
| 4 | 2 | 2 | 0.27940 | ~~1B2~~ | 9h |
| 5 | 1 | 2 | 0.37473 | 1A2 | ~~9h~~ |
| 6 | 1 | 3 | 0.48168 | 1A3 | 9h |
| 7 | 3 | 2 | 0.62620 | 1C2 | 12 |
| 8 | 3 | 3 | 0.84572 | 1C3 | 12 |
| 9 | 3 | 1 | 0.87154 | 1C1 | 12 |

Dosing for bottom injection is for 12h only. Extra bag from 9h will be used, but removed at 12 h. KW 7 SEPT12

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File Folder: 14a Item Number: 3 Page 1 of 30

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 JR

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 2 ***
Shawano - bottom injection Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.26233 | 2A3 | 6h |
| 2 | 3 | 1 | 0.28933 | 2C1 | 6h |
| 3 | 3 | 3 | 0.41823 | 2C3 | 6h |
| 4 | 1 | 1 | 0.56759 | 2A1 | 9h |
| 5 | 3 | 2 | 0.60252 | 2C2 | 9h |
| 6 | 1 | 2 | 0.63382 | 2A2 | 9h |
| 7 | 2 | 1 | 0.64069 | 2B1 | 12 |
| 8 | 2 | 3 | 0.80439 | 2B3 | 12 |
| 9 | 2 | 2 | 0.92279 | 2B2 | 12 |

* See note on page 1 of the randomization KW 7SEPT12

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Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 Jm

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 3 ***
Shawano - bottom injection Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 0.16703 | 3A1 | 6h |
| 2 | 3 | 3 | 0.19671 | 3C3 | 6h |
| 3 | 1 | 3 | 0.21867 | 3A3 | 6h |
| 4 | 2 | 3 | 0.23119 | 3B3 | 9h |
| 5 | 3 | 1 | 0.72793 | 3C1 | 9h |
| 6 | 1 | 2 | 0.80420 | 3A2 | 9h |
| 7 | 2 | 1 | 0.80905 | 3B1 | 12 |
| 8 | 3 | 2 | 0.88712 | 3C2 | 12 |
| 9 | 2 | 2 | 0.96360 | 3B2 | 12 |

* See note on page 1 of randomization JM 7SEP12

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 JAL

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 4 ***
Shawano - bottom injection Treatment

AEH-12-PSEUDO-04

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.06803 | 4A3 | 6h |
| 2 | 1 | 1 | 0.08714 | 4A1 | 6h |
| 3 | 2 | 1 | 0.26140 | 4B1 | 6h |
| 4 | 3 | 2 | 0.28491 | 4C2 | 9h |
| 5 | 3 | 1 | 0.45781 | 4C1 | 9h |
| 6 | 2 | 3 | 0.46232 | 4B3 | 9h |
| 7 | 1 | 2 | 0.47420 | 4A2 | 12 |
| 8 | 2 | 2 | 0.64459 | 4B2 | 12 |
| 9 | 3 | 3 | 0.74556 | 4C3 | 12 |

* See note on page 1 of randomization
JW
7SEP12

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Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 JAL

AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 5 ***
Shawano - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| ~~1~~ | ~~1~~ | ~~3~~ | ~~0.26233~~ | ① ~~2A3~~ | ~~6h~~ |
| ~~2~~ | ~~3~~ | ~~1~~ | ~~0.28933~~ | ~~2C1~~ | ~~6h~~ |
| ~~3~~ | ~~3~~ | ~~3~~ | ~~0.41823~~ | ~~2C3~~ | ~~6h~~ |
| ~~4~~ | ~~1~~ | ~~1~~ | ~~0.56759~~ | ~~2A1~~ | ~~9h~~ |
| ~~5~~ | ~~3~~ | ~~2~~ | ~~0.60252~~ | ~~2C2~~ | ~~9h~~ |
| ~~6~~ | ~~1~~ | ~~2~~ | ~~0.63382~~ | ~~2A2~~ | ~~9h~~ |
| 7 | 2 | 1 | 0.64069 | 2B1 | 12 |
| 8 | 2 | 3 | 0.80439 | 2B3 | 12 |
| 9 | 2 | 2 | 0.92279 | 2B2 | 12 |

* See note on page 1 of randomization
KW
7 SEP 12

① Tank numbers should be 5 not 2. KW 7SEP12
See Deviation #2 for further clarification.
KW
19NOV13

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 AEH-12-PSEUDO-04

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 6 ***
Shawano - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| ~~1~~ | ~~2~~ | ~~1~~ | ~~0.18641~~ | ~~6B1~~ | ~~6h~~ |
| ~~2~~ | ~~3~~ | ~~1~~ | ~~0.22996~~ | ~~6C1~~ | ~~6h~~ |
| ~~3~~ | ~~1~~ | ~~3~~ | ~~0.40217~~ | ~~6A3~~ | ~~6h~~ |
| ~~4~~ | ~~1~~ | ~~2~~ | ~~0.48348~~ | ~~6A2~~ | ~~9h~~ |
| ~~5~~ | ~~1~~ | ~~1~~ | ~~0.48881~~ | ~~6A1~~ | ~~9h~~ |
| ~~6~~ | ~~3~~ | ~~2~~ | ~~0.70464~~ | ~~6C2~~ | ~~9h~~ |
| 7 | 2 | 3 | 0.76432 | 6B3 | 12 |
| 8 | 3 | 3 | 0.93288 | 6C3 | 12 |
| 9 | 2 | 2 | 0.96790 | 6B2 | 12 |

* See note on page 1 of randomization KMW 7SEP12

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Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 7 ***
Shawano - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| ~~1~~ | ~~1~~ | ~~3~~ | ~~0.26233~~ | ~~2A3~~ | ~~6h~~ |
| ~~2~~ | ~~3~~ | ~~1~~ | ~~0.28933~~ | ~~2C1~~ | ~~6h~~ |
| ~~3~~ | ~~3~~ | ~~3~~ | ~~0.41823~~ | ~~2C3~~ | ~~6h~~ |
| ~~4~~ | ~~1~~ | ~~1~~ | ~~0.56759~~ | ~~2A1~~ | ~~9h~~ |
| ~~5~~ | ~~3~~ | ~~2~~ | ~~0.60252~~ | ~~2C2~~ | ~~9h~~ |
| 6 | 1 | 2 | 0.63382 | 2A2 | 9h |
| 7 | 2 | 1 | 0.64069 | 2B1 | 12 |
| 8 | 2 | 3 | 0.80439 | 2B3 | 12 |
| 9 | 2 | 2 | 0.92279 | 2B2 | 12 |

*See note on page 1 of randomization KLW 7SEP12

① Tank numbers should be 7 not 2 KLW 7SEP12 See Deviation #2 for further clarification. KLW 17NOV13

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 JAL

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 8 ***
Shawano - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 3 | 2 | 0.05681 | 8C2 | 6h |
| 2 | 1 | 2 | 0.07331 | 8A2 | 6h |
| 3 | 3 | 1 | 0.33534 | 8C1 | 6h |
| 4 | 3 | 3 | 0.41579 | 8C3 | 9h |
| 5 | 1 | 1 | 0.48937 | 8A1 | 9h |
| 6 | 1 | 3 | 0.54741 | 8A3 | 9h |
| 7 | 2 | 3 | 0.62872 | 8B3 | 12 |
| 8 | 2 | 2 | 0.64996 | 8B2 | 12 |
| 9 | 2 | 1 | 0.73310 | 8B1 | 12 |

* See note on page 1 of randomization. JAL 7SEP12

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12 JAL AEH-12-PSEUDO-04

Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical substrates
AEH-12-PSEUDO-04
Random assignment of substrate removal from tanks *** TANK 9 ***
Shawano - bottom injection Treatment

| Obs | row | position | x | tankn | trt |
|---|---|---|---|---|---|
| 1 | 1 | 3 | 0.26233 | (1) 2A3 | 6h |
| 2 | 3 | 1 | 0.28933 | 2C1 | 6h |
| 3 | 3 | 3 | 0.41823 | 2C3 | 6h |
| 4 | 1 | 1 | 0.56759 | 2A1 | 9h |
| 5 | 3 | 2 | 0.60252 | 2C2 | 9h |
| 6 | 1 | 2 | 0.63382 | 2A2 | 9h |
| 7 | 2 | 1 | 0.64069 | 2B1 | 12 |
| 8 | 2 | 3 | 0.80439 | 2B3 | 12 |
| 9 | 2 | 2 | 0.92279 | 2B2 | 12 |

* See note on page 1 of randomization KW 7SEP12

(1) Tank numbers should be 9 not 2. KW 7SEP12
See Deviation #2 for further clarification, KW 19NOV13

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AEH-12-PSEUDO-04

Analysis performed by J. Luoma SAS version 9.2 10:20 13AUG12

```
/*********************************************************************
*  Study Number  : AEH-12-PSUEDO-04
*  Study Director: Jim Luoma
*  date created  : 13 August 2012 - JAL
*  Verified by:  _______ (Date:_______)                page ____ of ____
*  Random allocation of treatment to tank.sas
*********************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=105 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
                       Substrate removal from tanks. See title below. Kw 28APR14
/*Random assignment of ~~treatment to experimental tanks~~*/
/*Location/exposure type: Shawano - bottom injection treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn = '1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
    run;
proc sort data=TANK1A;
 by x;
run;

data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
```

AEH-12-PSEUDO-04

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```
data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn =  2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
    if row = 2 and position = 1 then tankn = '2B1';
     if row = 2 and position = 2 then tankn = '2B2';
      if row = 2 and position = 3 then tankn = '2B3';
       if row = 3 and position = 1 then tankn = '2C1';
        if row = 3 and position = 2 then tankn = '2C2';
         if row = 3 and position = 3 then tankn = '2C3';
     run;
proc sort data=TANK2A;
 by x;
run;

data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;

data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
```

AEH-12-PSEUDO-04

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```
        if row = 3 and position = 1 then tankn = '3C1';
         if row = 3 and position = 2 then tankn = '3C2';
          if row = 3 and position = 3 then tankn = '3C3';
      run;
proc sort data=TANK3A;
 by x;
run;

data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;
proc sort data=TANK4A;
 by x;
run;

data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
```

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```
    if _n_ = 8 then trt = '12h ;
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK5A; set TANK5;
 if row = 1 and position = 1 then tankn = '5A1';
  if row = 1 and position = 2 then tankn = '5A2';
   if row = 1 and position = 3 then tankn = '5A3';
    if row = 2 and position = 1 then tankn = '5B1';
     if row = 2 and position = 2 then tankn = '5B2';
      if row = 2 and position = 3 then tankn = '5B3';
       if row = 3 and position = 1 then tankn = '5C1';
        if row = 3 and position = 2 then tankn = '5C2';
         if row = 3 and position = 3 then tankn = '5C3';
     run;
proc sort data=TANK5A;
 by x;
run;

data assign_trt_TANK5A; set TANK5A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h ;
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
```

```
run;
data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;
proc sort data=TANK6A;
 by x;
run;

data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn = '7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;
proc sort data=TANK7A;
 by x;
run;
```

```
data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;
proc sort data=TANK8A;
 by x;
run;

data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
```

```
run;
data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;
data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
     run;
proc sort data=TANK9A;
 by x;
run;

data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on artifical
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
```

8/17/12
JZ

AEH-12-PSEUDO-0?

```
*  date created  : 13 August 2012 - JAL
*  Verified by:  ______ (Date:______)                    page ____ of ____
*  Random allocation of treatment to tank.sas
*******************************************************************/
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Analysis performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=105 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

                          Substrate removal from tanks. See title on next page. 26APR14
/*Random assignment of ~~treatment to experimental tanks*~~/
/*Location/exposure type: Shawano - bottom injection treatment*/
data TANK1;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK1 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK1A; set TANK1;
 if row = 1 and position = 1 then tankn = '1A1';
  if row = 1 and position = 2 then tankn = '1A2';
   if row = 1 and position = 3 then tankn =  1A3';
    if row = 2 and position = 1 then tankn = '1B1';
     if row = 2 and position = 2 then tankn = '1B2';
      if row = 2 and position = 3 then tankn = '1B3';
       if row = 3 and position = 1 then tankn = '1C1';
        if row = 3 and position = 2 then tankn = '1C2';
         if row = 3 and position = 3 then tankn = '1C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK1.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK1A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.TANK1A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

```
data assign_trt_TANK1A; set TANK1A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h ;
  run;

NOTE: There were 9 observations read from the data set WORK.TANK1A.
NOTE: The data set WORK.ASSIGN_TRT_TANK1A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc print data= assign_trt_TANK1A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 1 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK1A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK2;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK2 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK2A; set TANK2;
 if row = 1 and position = 1 then tankn = '2A1';
  if row = 1 and position = 2 then tankn = '2A2';
   if row = 1 and position = 3 then tankn = '2A3';
```

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```
     if row = 2 and position = 1 then tankn = '2B1';
      if row = 2 and position = 2 then tankn = '2B2';
       if row = 2 and position = 3 then tankn = '2B3';
        if row = 3 and position = 1 then tankn = '2C1';
         if row = 3 and position = 2 then tankn = '2C2';
          if row = 3 and position = 3 then tankn = '2C3';
      run;

NOTE: There were 9 observations read from the data set WORK.TANK2.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK2A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.TANK2A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



data assign_trt_TANK2A; set TANK2A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK2A.
NOTE: The data set WORK.ASSIGN_TRT_TANK2A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
! artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 2 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;
```

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```
NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
```

```
     real time           0.00 seconds
     cpu time            0.00 seconds


data TANK3;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK3 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data TANK3A; set TANK3;
 if row = 1 and position = 1 then tankn = '3A1';
  if row = 1 and position = 2 then tankn = '3A2';
   if row = 1 and position = 3 then tankn = '3A3';
    if row = 2 and position = 1 then tankn = '3B1';
     if row = 2 and position = 2 then tankn = '3B2';
      if row = 2 and position = 3 then tankn = '3B3';
       if row = 3 and position = 1 then tankn = '3C1';
        if row = 3 and position = 2 then tankn = '3C2';
         if row = 3 and position = 3 then tankn = '3C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK3.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
     real time           0.03 seconds
     cpu time            0.03 seconds


proc sort data=TANK3A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.TANK3A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data assign_trt_TANK3A; set TANK3A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
```

```
if _n_ = 5 then trt = '9h';
 if _n_ = 6 then trt = '9h';
  if _n_ = 7 then trt = '12h';
   if _n_ = 8 then trt = '12h';
if _n_ = 9 then trt = '12h';
 run;

NOTE: There were 9 observations read from the data set WORK.TANK3A.
NOTE: The data set WORK.ASSIGN_TRT_TANK3A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK3A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 3 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK3A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK4;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK4 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK4A; set TANK4;
 if row = 1 and position = 1 then tankn = '4A1';
  if row = 1 and position = 2 then tankn = '4A2';
   if row = 1 and position = 3 then tankn = '4A3';
    if row = 2 and position = 1 then tankn = '4B1';
     if row = 2 and position = 2 then tankn = '4B2';
      if row = 2 and position = 3 then tankn = '4B3';
       if row = 3 and position = 1 then tankn = '4C1';
        if row = 3 and position = 2 then tankn = '4C2';
         if row = 3 and position = 3 then tankn = '4C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK4.
```

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```
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK4A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.TANK4A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



data assign_trt_TANK4A; set TANK4A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK4A.
NOTE: The data set WORK.ASSIGN_TRT_TANK4A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK4A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 4 ***';
title4 h=1 'Shawaro - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK4A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK5;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
```

```
   end;
  end;
 run;

NOTE: The data set WORK.TANK5 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


 data TANK5A; set TANK5;
  if row = 1 and position = 1 then tankn = '5A1';
   if row = 1 and position = 2 then tankn = '5A2';
    if row = 1 and position = 3 then tankn = '5A3';
     if row = 2 and position = 1 then tankn = '5B1';
      if row = 2 and position = 2 then tankn = '5B2';
       if row = 2 and position = 3 then tankn = '5B3';
        if row = 3 and position = 1 then tankn = '5C1';
         if row = 3 and position = 2 then tankn = '5C2';
          if row = 3 and position = 3 then tankn = '5C3';
      run;

NOTE: There were 9 observations read from the data set WORK.TANK5.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


 proc sort data=TANK5A;
  by x;
 run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.TANK5A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



 data assign_trt_TANK5A; set TANK5A;
  if _n_ = 1 then trt = '6h';
   if _n_ = 2 then trt = '6h';
    if _n_ = 3 then trt = '6h';
     if _n_ = 4 then trt =  9h';
  if _n_ = 5 then trt = '9h';
   if _n_ = 6 then trt = '9h';
    if _n_ = 7 then trt = '12h';
     if _n_ = 8 then trt = '12h';
  if _n_ = 9 then trt = '12h';
   run;

NOTE: There were 9 observations read from the data set WORK.TANK5A.
NOTE: The data set WORK.ASSIGN_TRT_TANK5A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
```

```
     real time           0.01 seconds
     cpu time            0.01 seconds
```

AEH-12-PSEUDO-04

```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 5 ***';
title4 h=1 'Shawano - botton injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data TANK6;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK6 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


data TANK6A; set TANK6;
 if row = 1 and position = 1 then tankn = '6A1';
  if row = 1 and position = 2 then tankn = '6A2';
   if row = 1 and position = 3 then tankn = '6A3';
    if row = 2 and position = 1 then tankn = '6B1';
     if row = 2 and position = 2 then tankn = '6B2';
      if row = 2 and position = 3 then tankn = '6B3';
       if row = 3 and position = 1 then tankn = '6C1';
        if row = 3 and position = 2 then tankn = '6C2';
         if row = 3 and position = 3 then tankn = '6C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK6.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
     real time           0.01 seconds
     cpu time            0.01 seconds


proc sort data=TANK6A;
 by x;
run;
```

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```
NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.TANK6A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data assign_trt_TANK6A; set TANK6A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h ;
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK6A.
NOTE: The data set WORK.ASSIGN_TRT_TANK6A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK6A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 6 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK6A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data TANK7;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK7 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

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```
data TANK7A; set TANK7;
 if row = 1 and position = 1 then tankn = '7A1';
  if row = 1 and position = 2 then tankn = '7A2';
   if row = 1 and position = 3 then tankn = '7A3';
    if row = 2 and position = 1 then tankn = '7B1';
     if row = 2 and position = 2 then tankn = '7B2';
      if row = 2 and position = 3 then tankn = '7B3';
       if row = 3 and position = 1 then tankn = '7C1';
        if row = 3 and position = 2 then tankn = '7C2';
         if row = 3 and position = 3 then tankn = '7C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK7.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK7A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.TANK7A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



data assign_trt_TANK7A; set TANK7A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK7A.
NOTE: The data set WORK.ASSIGN_TRT_TANK7A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

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```
proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 7 ***';
title4 h=1 'Shawano - botton injection Treatment ';
```

```
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```



```
data TANK8;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK8 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK8A; set TANK8;
 if row = 1 and position = 1 then tankn = '8A1';
  if row = 1 and position = 2 then tankn = '8A2';
   if row = 1 and position = 3 then tankn = '8A3';
    if row = 2 and position = 1 then tankn = '8B1';
     if row = 2 and position = 2 then tankn = '8B2';
      if row = 2 and position = 3 then tankn = '8B3';
       if row = 3 and position = 1 then tankn = '8C1';
        if row = 3 and position = 2 then tankn = '8C2';
         if row = 3 and position = 3 then tankn = '8C3';
     run;

NOTE: There were 9 observations read from the data set WORK.TANK8.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort data=TANK8A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.TANK8A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

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```

data assign_trt_TANK8A; set TANK8A;
 if _n_ = 1 then trt = '6h';
```

```
   if _n_ = 2 then trt = '6h';
    if _n_ = 3 then trt = '6h';
     if _n_ = 4 then trt = '9h';
  if _n_ = 5 then trt = '9h';
   if _n_ = 6 then trt = '9h';
    if _n_ = 7 then trt = '12h';
     if _n_ = 8 then trt = '12h';
  if _n_ = 9 then trt = '12h';
   run;

NOTE: There were 9 observations read from the data set WORK.TANK8A.
NOTE: The data set WORK.ASSIGN_TRT_TANK8A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK8A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 8 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK8A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data TANK9;
do row = 1 to 3 by 1;
 do position = 1 to 3 by 1;
  x = ranuni(-1);
  output;
  end;
 end;
run;

NOTE: The data set WORK.TANK9 has 9 observations and 3 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


data TANK9A; set TANK9;
 if row = 1 and position = 1 then tankn = '9A1';
  if row = 1 and position = 2 then tankn = '9A2';
   if row = 1 and position = 3 then tankn = '9A3';
    if row = 2 and position = 1 then tankn = '9B1';
     if row = 2 and position = 2 then tankn = '9B2';
      if row = 2 and position = 3 then tankn = '9B3';
       if row = 3 and position = 1 then tankn = '9C1';
        if row = 3 and position = 2 then tankn = '9C2';
         if row = 3 and position = 3 then tankn = '9C3';
```

AEH-12-PSEUDO-04

```
      run;

NOTE: There were 9 observations read from the data set WORK.TANK9.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


proc sort data=TANK9A;
 by x;
run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.TANK9A has 9 observations and 4 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data assign_trt_TANK9A; set TANK9A;
 if _n_ = 1 then trt = '6h';
  if _n_ = 2 then trt = '6h';
   if _n_ = 3 then trt = '6h';
    if _n_ = 4 then trt = '9h';
 if _n_ = 5 then trt = '9h';
  if _n_ = 6 then trt = '9h';
   if _n_ = 7 then trt = '12h';
    if _n_ = 8 then trt = '12h';
 if _n_ = 9 then trt = '12h';
  run;

NOTE: There were 9 observations read from the data set WORK.TANK9A.
NOTE: The data set WORK.ASSIGN_TRT_TANK9A has 9 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data= assign_trt_TANK2A;
title1 h=2 'Efficacy of Pseudomonas fluorescens (Pf-CL145A)for controlling zebra mussesl on
! artifical substrates';
title2 h=1.5 'AEH-12-PSEUDO-04';
title3 h=1 'Random assignment of substrate removal from tanks *** TANK 9 ***';
title4 h=1 'Shawano - bottom injection Treatment ';
run;

NOTE: There were 9 observations read from the data set WORK.ASSIGN_TRT_TANK2A.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

8/12/12

FF # HA
Item No. 3
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```
NOTE: This SAS session is using a registry in WORK.  All changes will be lost at the end of this sess
```

# Appendix 4. Test Article Information

| Item Number | Item Description | Number of Pages | Report Page Number |
|---|---|---|---|
| 1 | Material Safety Data Sheet: MBI-401 SDP | 2 | 240 |
| 2 | MBI-401 SDP (lots # 401P12163C and 401P12164C) Test Article: UPS Next Day Air Label (shipped to Jim Luoma) | 1 | 242 |
| 3 | MBI-401 SDP (lot # 401P12163C) Test Article: Certificate of Analysis | 1 | 243 |
| 4 | MBI-401 SDP (lot # 401P12164C) Test Article: Certificate of Analysis | 1 | 244 |
| 5 | "Test Chemical Stock Preparation Data Form" Datasheet (Lake Carlos) | 1 | 245 |
| 6 | "Test Chemical Stock Preparation Data Form" Datasheet (Lake Shawano) | 1 | 246 |
| 7 | FedEx Priority Overnight Airbill (shipped to Denise Mayer) for post-treatment product validation – dated January 8, 2013 | 1 | 247 |
| 8 | NYSM Post –Treatment Product Validation Assay MBI-401 SDP lot #(s) 401P12163C and 401P12164C Mix | 3 | 248 |
| 9 | Copy of test article log book for MBI-401 SDP; lot #(s) 401P12163C and 401P12164C Mix; Container 1 of 6 (Used for Lake Carlos) | 5 | 251 |
| 10 | Copy of test article log book for MBI-401 SDP; lot #(s) 401P12163C and 401P12164C Mix; Container 2 of 6 (Used for Lake Carlos) | 4 | 256 |
| 11 | Copy of test article log book for MBI-401 SDP; lot #(s) 401P12163C and 401P12164C Mix; Container 3 of 6 (Used for Lake Shawano) | 4 | 260 |
| 12 | Copy of test article log book for MBI-401 SDP; lot #(s) 401P12163C and 401P12164C Mix; Container 4 of 6 (Used for Lake Shawano) | 4 | 264 |
| 13 | Copy of test article log book for MBI-401 SDP; lot #(s) 401P12163C and 401P12164C Mix; Container 6 of 6 (Used for Lake Carlos water analysis by RMB Environmental Laboratories) | 4 | 268 |

AEH-12-PSEUDO-04

# MATERIAL SAFETY DATA SHEET



**Product Name: MBI-401 SDP**

Trade names/ Synonyms: MBI-401 SDP
EPA Registration Number: None, Experimental
PMRA Research Authorization #: 0030-RP-10
Primary Hazards: Inhalation

Contact: Marrone Bio Innovations, 2121 Second Street, Suite B-107, Davis, CA 95618
Phone (Business hours): 530-750-2800
www.marronebioinnovations.com
For emergencies such as leaks or spills call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300

SECTION 1: MATERIAL IDENTIFICATION

INGREDIENT 1

| | |
|---|---|
| Common Name: | CL145A strain of *Pseudomonas fluorescens* |
| Chemical Name: | Not applicable |
| Molecular Formula: | Not applicable |
| CAS Number: | Not applicable |
| Percent: | 50% |

OTHER INGREDIENTS: inert, non-reactive

SECTION 2: PHYSICAL DATA

| | |
|---|---|
| Boiling Point: | Not applicable |
| Melting Point: | Not applicable |
| Bulk Density: | 0.78 g/ml |
| Solubility in Water: | Dispersible in water |
| Appearance: | Powder |
| Color: | Tan |
| Odor: | Sweet, musty |

SECTION 3: FIRE AND EXPLOSION DATA

| | |
|---|---|
| Flash Point: | Not flammable |
| Method: | Not applicable |
| Extinguishing Media: | Use extinguishing media appropriate for the surrounding fire |
| Special Fire Fighting Procedures: | None |
| Unusual Fire and Explosion Hazards: | None |

SECTION 4: REACTIVITY

| | |
|---|---|
| Stability: | Material is non-reactive |
| Hazardous Polymerization: | Does not occur |
| Incompatibility: | None known |
| Hazardous Decomposition Products: | None known |
| Conditions to avoid: | None known |

SECTION 5: HEALTH HAZARDS

| | |
|---|---|
| Primary Route of Entry: | Skin contact, Eye, Inhalation |
| Exposure Limit: | Not established |
| Corrosive: | Not corrosive |
| Inhalation: | May be irritating to respiratory tract for some individuals. Avoid breathing dust. |
| Skin/ Eye Irritation: | May be irritating to skin and eyes for some individuals. |
| Effects of Overexposure: | If product comes in contact with eyes or skin, irritation may occur. |
| Toxicity: | None of the components of this product are listed as carcinogenic by NTP, IARC, OSHA |

Acute studies:

| | |
|---|---|
| Acute Oral $LD_{50}$ (Rat): | >5,000 mg/kg (very low toxicity) |
| Acute Dermal $LD_{50}$ (Rabbit) | >2,000 mg/kg (non-irritating, mild or slight irritation) |
| Primary Dermal Irritation | Slight Irritation, Class 4 |
| Eye Irritation | Minimal Irritation, Class 4 |
| Inhalation: | >2.25 g/ml, Class 4 |

SECTION 6: FIRST AID

Emergency First Aid Procedures:

If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center of doctor for treatment advice.

If inhaled: Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.

If on skin: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for further treatment advice.

If swallowed: Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to by a poison control center or doctor. Do not give anything by mouth to an unconscious person.

File Folder: 7

Item Number: 4

# MATERIAL SAFETY DATA SHEET

MBI-401 Spray Dried Powder, August 2010 — Page 2 of 2

## SECTION 7: SPILL, LEAK AND DISPOSAL PROCEDURES

Steps to be taken in case material is released or spilled:

Wear suitable protective clothing such as long-sleeved shirt, pants, waterproof gloves and shoes with socks. Carefully mop or sweep up spill and place in a closed container for disposal.

Waste disposal method: Dispose of in accordance with all applicable federal, state, and local environmental regulations.

For emergencies such as leaks or spills, call CHEMTREC 24-hour toll-free hotline at 1.800.424.9300.

## SECTION 8: SPECIAL HANDLING

| | |
|---|---|
| Respiratory: | Use a NIOSH approved respirator with any N-95, P-95, R-95 or HE filter for biological products when mixing/loading the product. |
| Protective gloves: | Wear gloves made of Latex or other impervious material. |
| Eye protection: | Safety goggles or safety glasses with side shields recommended. |
| Other protective clothes: | Clothing to prevent prolonged skin contact as needed such as long-sleeved shirt, long pants and shoes with socks. |

## SECTION 9: SPECIAL PRECAUTIONS

Precautions to be taken in handling and storing:

Use a NIOSH approved respirator with any N-95, P-95, R-95 or HE filter for biological products when mixing/loading the product.

Store in a dry area inaccessible to children. Store in original containers only. Keep container closed when not in use.

Empty container completely and dispose of in accordance with all applicable federal, state, and local environmental regulations.

Wash any contamination from skin or eyes immediately. Wash hands and exposed skin before eating, drinking, smoking after work or using the toilet.

## SECTION 10: SHIPPING REGULATIONS

| | |
|---|---|
| Proper shipping name: | None |
| DOT Label (s) Required: | None |
| Freight Classification: | Insecticides, Fungicides N.O.I., Other Than Poisons. NMFC 102120 Class 60 |

SARA Title III Hazard Classification:

| | |
|---|---|
| Immediate (acute) Health: | None |
| Delayed (chronic) Health: | None |
| Fire: | None |
| Sudden Release of Pressure: | None |
| Reactivity: | None |

National Fire Protection Association Rating:

| | |
|---|---|
| Health: | None |
| Flammability: | None |
| Reactivity: | None |

This document set forth is based on information that Marrone Bio Innovations, Inc. (MBI) believes to be accurate. No warranty, expressed or implied, is intended. The information is provided solely for your information and consideration and MBI assumes no legal responsibility for use or reliance thereon.

Marrone™ Bio Innovations

File Folder: 7

Item Number: 4

THE STATE EDUCATION DEPARTMENT / OFFICE OF CULTURAL EDUCATION

New York State Museum Field Research Laboratory
51 Fish Hatchery Road
Cambridge, NY 12816
Tel. 518-677-8245
Fax 518-677-5236
E-mail: dmayer@mail.nysed.gov

AEH-12-PSEUDO-04

**PACKING LIST**
**DATE OF SHIPMENT - 2012/08/06**

Ship from:
Denise Mayer
New York State Museum
Field Research Laboratory
51 Fish Hatchery Road
Cambridge, NY 12816

Ship To:
Jim Luoma
USGS UMESC
2630 Fanta Reed Road
La Crosse, WI 54603

Shipping Method: UPS Next Day Air
Required by: 2012/08/07

Item Description:

MBI-401 SDP Lot # 401P12163C and 401P12164C Mix in six containers of approximately 0.5 Kg each (total weight 3 Kg):

Packaged by D. Mayer.
Shipped on ice. Store at 4°C, protected from light.

Manufactured: 06-21-2012
Expiration date 06-21-2013

File Folder: 7 Item Number: 1 **Page** 1 **of** 1

Marrone™ Bio Innovations

AEH-12-PSEUDO-04

**CERTIFICATE OF ANALYSIS**

| | |
|---|---|
| Name of Product: | MBI-401 SDP |
| Active Ingredient: | 100% *Pseudomonas fluorescens* strain CL145A cells and spent fermentation media |
| Percent Active Ingredient: | 50% by weight |
| Viable Cfu/g: | 0 cfu/g *Pseudomonas fluorescens* strain CL145A |
| Lot Number: | 401P12163C |
| Mussel Bioassay: | Pass |
| Appearance: | Tan powder |
| Storage Conditions: | 4 °C, protected from light |
| Date of Manufacture: | 21 June 2012 |
| Expiration Date: | 21 June 2013 |

I hereby certify that the above information is true and correct.

Quality Control: [redacted] Date: 01 August 2012

Tamara Nicholson, Quality Control Supervisor

2121 Second Street, Suite B-107 • Davis, CA 95618 • Phone: 530-750-2800

File Folder: 7 Item Number: ~~1~~2 wrong item number. 2 is correct. TRS 1/27/14 Page 1 of 1

Marrone™ Bio Innovations

AEH-12-PSEUDO-04

# CERTIFICATE OF ANALYSIS

| | |
|---|---|
| Name of Product: | MBI-401 SDP |
| Active Ingredient: | 100% *Pseudomonas fluorescens* strain CL145A cells and spent fermentation media |
| Percent Active Ingredient: | 50% by weight |
| Viable Cfu/g: | 0 cfu/g *Pseudomonas fluorescens* strain CL145A |
| Lot Number: | 401P12164C |
| Mussel Bioassay: | Pass |
| Appearance: | Tan powder |
| Storage Conditions: | 4 °C, protected from light |
| Date of Manufacture: | 21 June 2012 |
| Expiration Date: | 21 June 2013 |

I hereby certify that the above information is true and correct.

Quality Control: [redacted] Date: 01 August 2012

Tamara Nicholson, Quality Control Supervisor

2121 Second Street, Suite B-107 • Davis, CA 95618 • Phone: 530-750-2800

File Folder: 7 Item Number: 3 Page 1 of 1

Study Number: AEH-12-PSEUDO-04 File Folder: 7 Lab book/pgs: E-log pages 11 98 Reviewed by: TS Date: 16JAN14 Verified by: JR Date: 2/11/14

# Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluroescens* strain 145A

Test Chemical Lot #: 401P12163C and 401P12164C Mix Date Rec'd 7-Aug-12 Exp. Date 21-Jun-12

Test Organism : zebra mussels Test Location: Lake Carlos, Alexandria, MN

Instruments Used: Mettler Toledo PG 2002-S S/N 1118470288
Sartorius CPA 225D S/N 21450930

**Weights of Chemical Samples:**

| Sample ID | Sample Weight | Comments | Date | Initials |
|---|---|---|---|---|
| Analytical #1 | 2.00007g | whole water body KW 15AUG12 | 12AUG12 | KW |
| Analytical #2 | 2.00035g | | ↑ | ↑ |
| Stock #1 | 35.00g | | | |
| Stock #2 | 35.03g | | | |
| Stock #3 | 35.04g | | | |
| Stock #4 | 70.01g | | | |
| Stock #5 | 70.00g | | | |
| Stock #6 | 70.04g | | | |
| Stock #7 | 75.01g | | ↓ | ↓ |
| Stock #8 | 150.05g | | 12AUG12 | KW |

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

**Stock Solution Preparation:**

| Sample ID | Dilution Volume (mL) | Dilution Time | Use | Exposure Time | Date | Initials |
|---|---|---|---|---|---|---|
| Analytical #1 | 500 | 0800 | Used to make dilutions for standard curve, whole body | — | 15AUG12 | KW |
| Stock #1 | 8000 | ① 0945 | Tank 1 whole body | 0945 | ↑ | ↑ |
| Stock #2 | 8000 | ① — | Tank 8 whole body | 0954 | | |
| Stock #3 | 8000 | ① — | Tank 4 whole body | 0958 | | |
| Stock #4 | 8000 | ① — | Tank 9 whole body | 1007 | | |
| Stock #5 | 8000 | ① — | Tank 7 whole body | 1010 | ↓ | ↓ |
| Stock #6 | 8000 | ① — | Tank 6 whole body | 1015 | 15AUG12 | KW |
| Analytical #2 | 500 | 0830 | Used to make dilutions for standard curve for bioassay injection | — | 17AUG12 | KW |
| Stock #8 | 2500 | 0940 | 100mg/L tanks | ② | 17AUG12 | KW |
| Stock #7 | 2500 | 1015 | 50mg/L tanks | ② | 17AUG12 | KW |

① Stock was not diluted. Mixed with tank water and poured back into tank. KW 15AUG12

② Exposure Times
Tank 9 - 1008
Tank 2 - 1021
Tank 5 - 1030
Tank 4 - 1046
Tank 8 - ~~1100~~ 1056 write over KW 17AUG12
Tank 1 - 1106

Item Number: 5 Page 1 of 1

Study Number: AEH-12-PSEUDO-04 Reviewed by: TTS Date: 16JAN14
File Folder: 7 Lab book/pgs: E-log Pages 20-21 Verified by: JCL Date: 2/11/14

# Test Chemical Stock Preparation Data Form

Test Chemical: *Pseudomonas fluroescens* strain 145A
Test Chemical Lot #: 401P12163C and 401P12164C Mix Date Rec'd 7-Aug-12 Exp. Date 21-Jun-12
Test Organism : zebra mussels Test Location: Shawano
Instruments Used: Mettler Toledo PG 2002-S S/N 1118470288
Sartorius CPA225D S/N 26450930

**Weights of Chemical Samples:**

| Sample ID | Sample Weight | Comments | Date | Initials |
|---|---|---|---|---|
| Analytical #1 | 2.00072g | | 27Aug12 | KW |
| Analytical #2 | 2.00029g | | ↑ | ↑ |
| Stock #1 | 35.10g | | | |
| Stock #2 | 35.06g | | | |
| Stock #3 | 35.07g | | | |
| Stock #4 | 70.02g | | | |
| Stock #5 | 70.04g | | | |
| Stock #6 | 70.02g | | | |
| Stock #7 | 60.06g | | ↓ | ↓ |
| Stock #8 | 120.07g | | 27Aug12 | KW |

NOTE: Chemical samples to be stored refrigerated until used for stock preparation.

**Stock Solution Preparation:**

| Sample ID | Dilution Volume (mL) | Dilution Time | Use | Exposure Time | Date | Initials |
|---|---|---|---|---|---|---|
| Analytical #1 | 500 | 0800 | standard curve for whole tank | Whole body | 6SEP12 | KW |
| Stock #1 | 8000 | 0855 | Tank 2 whole body | 0900 | ↑ | ↑ |
| Stock #2 | 8000 | 0902 | Tank 8 whole body | 0905 | | |
| Stock #3 | 8000 | 0906 | Tank 6 whole body | 0909 | | |
| Stock #4 | 8000 | 0913 | Tank 9 whole body | 0915 | | |
| Stock #5 | 8000 | 0917 | Tank 3 whole body | 0920 | ↓ | ↓ |
| Stock #6 | 8000 | 0922 | Tank 6 whole body | 0925 | 6SEP12 | KW |
| Analytical #2 | 500 | 0745 | standard curve for bottom | bottom injection | 8SEP12 | KW |
| Stock #7 | 12000 | 0809 | 50mg/L tanks | 0841-0901 | ↕ | ↕ |
| Stock #8 | 12000 | 0900 | 100mg/L tanks | 0907-0939 | 8SEP12 | KW |

Item Number: 7 Page 1 of 1

AEH-12-PSEUDO-04

FedEx Express NEW Package US Airbill

FedEx Tracking Number 8008 0133 0994

Form ID No. 0215

Sender's Copy

**1 From** *Please print and press hard.*

Date 8 JAN 2013 Sender's FedEx Account Number 1201-0006-6

Sender's Name Kerry Weber Phone (608) 783-6451

Company UPPER MIDWEST ENVIRO SCIENCE

Address 2630 FANTA REED RD Dept./Floor/Suite/Room

City LA CROSSE State WI ZIP 54603-1223

**2 Your Internal Billing Reference** First 24 characters will appear on invoice. OPTIONAL

**3 To**

Recipient's Name Denise Mayer Phone (518) 677-8245

Company New York State Museum

Address Field Research Laboratory

We cannot deliver to P.O. boxes or P.O. ZIP codes. Dept./Floor/Suite/Room

Address 51 Fish Hatchery Road

Use this line for the HOLD location address or for continuation of your shipping address.

City Cambridge State NY ZIP 12816

☐ HOLD Weekday FedEx location address REQUIRED. NOT available for FedEx First Overnight.

☐ HOLD Saturday FedEx location address REQUIRED. Available ONLY for FedEx Priority Overnight and FedEx 2Day to select locations.

0453538510

The FedEx US Airbill has changed. See Section 4.
For shipments over 150 lbs. – order the new FedEx Express Freight US Airbill.

**4 Express Package Service** *To most locations.

NOTE: Service order has changed. Please select carefully.

Packages up to 150 lbs. For packages over 150 lbs., use the new FedEx Express Freight US Airbill.

Next Business Day

☐ FedEx First Overnight — Earliest next business morning delivery to select locations. Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

☒ FedEx Priority Overnight — Next business morning.* Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

☐ FedEx Standard Overnight — Next business afternoon.* Saturday Delivery NOT available.

2 or 3 Business Days

☐ NEW FedEx 2Day A.M. — Second business morning.* Saturday Delivery NOT available.

☐ FedEx 2Day — Second business afternoon.* Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.

☐ FedEx Express Saver — Third business day.* Saturday Delivery NOT available.

**5 Packaging** *Declared value limit $500.

☐ FedEx Envelope* ☐ FedEx Pak* ☐ FedEx Box ☐ FedEx Tube ☒ Other

**6 Special Handling and Delivery Signature Options**

☐ SATURDAY Delivery — NOT available for FedEx Standard Overnight, FedEx 2Day A.M., or FedEx Express Saver.

☐ No Signature Required — Package may be left without obtaining a signature for delivery.

☐ Direct Signature — Someone at recipient's address may sign for delivery. Fee applies.

☐ Indirect Signature — If no one is available at recipient's address, someone at a neighboring address may sign for delivery. For residential deliveries only. Fee applies.

Does this shipment contain dangerous goods? One box must be checked.

☒ No ☐ Yes As per attached Shipper's Declaration. ☐ Yes Shipper's Declaration not required. ☐ Dry Ice Dry Ice, 9, UN 1845 ____ x ____ kg

Dangerous goods (including dry ice) cannot be shipped in FedEx packaging or placed in a FedEx Express Drop Box. ☐ Cargo Aircraft Only

**7 Payment** *Bill to:*

Enter FedEx Acct. No. or Credit Card No. below.

☒ Sender Acct. No. in Section 1 will be billed. ☐ Recipient ☐ Third Party ☐ Credit Card ☐ Cash/Check

FedEx Acct. No. / Credit Card No. Exp. Date

Total Packages 1 Total Weight ____ lbs. Total Declared Value* $ ____ .00

*Our liability is limited to $100 unless you declare a higher value. See back for details. By using this Airbill you agree to the service conditions on the back of this Airbill and in the current FedEx Service Guide, including terms that limit our liability.

611

Rev. Date 11/10 • Part #163134 • ©1994–2010 FedEx • PRINTED IN U.S.A. SRS

PULL AND RETAIN THIS COPY BEFORE AFFIXING TO THE PACKAGE. NO POUCH NEEDED.

File Folder: 7 Item Number: 8 Page 1 of 1

# NYSM Post-Treatment Product Validation Assay

MBI-401 SDP 401P12163C and 401P12164C Mix

(USGS Study #AEH-~~13~~-PSEUDO-04 Field trials at Shawano and Carlos in 2012)

Incorrect study number. Correct study number is AEH-12-PSEUDO-04. FWW 27JAN2014

Date product received from USGS: 2013/01/09

Date of start of test: 2013/01/09

AEH-12-PSEUDO-04

**BACKGROUND:** As standard protocol for the USEPA project, each time a batch of Zequanox product is used in a test a UMESC, a portion of the product is bioassayed by the NYSM to validate toxicity post-treatment.

- MBI-401 SDP 401P12163C and 401P12164C Mix
    - USGS Study #AEH-~~13~~- PSEUDO-04 Field trials at Shawano and Carlos in 2012

      Incorrect study number. Correct study number is AEH-12-PSEUDO-04 FWW 27JAN2014

**PURPOSE:** Post-test product validation of MBI-401 SDP 401P12163C and 401P12164C Mix used in AEH-13-PSEUDO-04 Field Trials at lakes Shawano and Carlos.

**MATERIALS AND METHODS:**

Preparation of product for testing:

Product was shipped under cold conditions and held in the laboratory refrigerator at 4°C until use. Within 30 min of treatment application, prepare each at treatment stock of each MBI-401 formulated product:

**MBI-401 SDP 401P12163C and 401P12164C Mix (SDP – 50% active ingredient):** 3 g of the powder from each sample was added slowly to a beaker with dilution water with stirring on a stir plate for even suspension and then the total volume was adjusted to 30 ml with dilution water. The suspension was transferred to a 50 ml centrifuge and stored in refrigerator until ready to use. The suspension, when evenly dispersed was 100 mg product/ml or 50 mg a.i./ml. For 200 ppm a.i. treatments in testing jars, 2 ml were added to each testing jar (500 ml).

**Cambridge CF (Standard for Positive Controls):**

As an efficacy standard, we used *Pf*-CL145A killed CF that was maintained at -80°C (Cambridge CF). Since its production in 2005, this material has been valuable as a reference standard. The Cambridge CF was produced in 2005 (2005-0027) in 100-L batches 10, 11 and 12 and E-beamed to kill the cells. The solution, at 110 mg/ml dry weight, is stored in 1 cm thick sheets in the Cambridge ultrafreezer at -80°C. A section of the sheet was broken off and weighed to determine volume (ca. 1 g = 1 ml).

For this bioassay, a positive control suspension was produced on 06/18/2012 from the frozen blocks described above and dispensed into multiple 50-ml centrifuge tubes for single-use treatment of bioassays. The dry weight of the material was 68.15 mg/ml; therefore 1.5 ml was added to each testing jar to treat at 200 ppm.

Mussel collections:

Mussels were scraped from substrates (rocks) in the field and brought back to the lab in coolers. Mussels were held unchlorinated water in aquaria with aeration and filtration (Whisper filters) at 10±2°C. Approximately one week before the test, jars of mussels were placed in aquaria containing 10±2°C tap water with low aeration in the laboratory, wrapped in towels to slow warming, and allowed to warm to ambient laboratory temperature (20±1°C), after which filtration and high aeration was applied to the aquarium. One day prior to treatment exposure, mussels were carefully examined and placed into testing jars and allowed to attach overnight at ambient laboratory temperature (ca. 20°C).

File Folder: 7 Item Number: ~~79~~ wrong item# FWW 29APR14 Page 1 of 3

Mussel collection and handling:

| Species | Collection site | Collection date | Date in lab (20°C) | Picked for test |
|---|---|---|---|---|
| Zebra mussels | Hedges Lake (Washington County) | 11/07/2012 | 01/02/2013 | 01/08/2013 |

Experimental design:
For validation of efficacy the following treatments will be set up:

Zebra mussels (25 mussels/jar):
3 – Untreated Control (A, B, C)
3 – 200 ppm (a.i.) Cambridge CF Positive Control (*Pf*-CL145A killed cells) (A, B, C)
3 – 200 ppm (a.i.) 401P12163C and 401P12164C Mixed (A, B, C)

Total of 9 testing jars.

Testing jar bioassay protocol:
On the day prior to treatment (01/08/2013) mussels were carefully examined and 25 mussels placed into each testing jar containing ca. 100 ml aerated hard water and allowed to attach overnight. The next morning (01/09/2013), unattached mussels were removed and replaced with attached mussels from an extra glass Petri dish. Water was replaced with 500 ml fresh aerated hard water.

After at least one hour, the treatment was applied. The optical density of each jar was measured in duplicate ($A_{660\ nm}$ Genesys Spectrophotometer).

After 24 hr of treatment, mussel mortality was checked and mussels were transferred to square plastic dishes with fresh aerated hard water. Mortality was checked and recorded each day with water replacements, for an additional 7 days (8 days total). On the final day of mortality checks, 20 mussels were measured from the untreated controls using a caliper.

**Results:**

Mussel length: Zebra mussels 19.65 ± 2.36 mm.

Optical density of treatments:

| Treatment | Mean (±SD) OD ($A_{660\ nm}$) |
|---|---|
| Untreated Control | 0.001 ± 0.001 |
| Cambridge CF (Positive Control) | 0.171 ± 0.010 |
| MBI-401 SDP 401P12163C and 401P12164C Mixed | 0.226 ± 0.004 |

Zebra mussel mortality: Mussels were treated in triplicate testing jars (500 ml) at 20°C for 24 hr and mortality was recorded for a total of 55 days.

| Treatment | Mean % mortality (±SD) |
|---|---|
| Untreated Control | 0.0 ± 0.0% |
| Cambridge CF (Positive Control) | 73.3 ± 8.3% |
| MBI-401 SDP 401P12163C and 401P12164C Mixed | 70.7 ± 4.6% |

2013-0001 MBI-401 SDP 401P12163C and 64C Mix Post-test from 2012 field trials Validation



**MBI-401 SDP 401P12163C and 401P12164C Mixed PASSED the post-test bioassay validation (71% mortality). Untreated control mortality was 0%.**

File Folder: 7

Item Number: 79 wrong item # KWS 29APR14 Page 3 of 3

AEH-12-PSEUDO-04

# CHEMICAL LOG BOOK

T OF THE INTERIOR
TY SEAL
TAMPER!

## MBI-401 SDP

### Marrone Bio Innovations
### Davis, California

### LOT NUMBERS:
### 401P12163C
### and
### 401P12164C
### (Received Mixed in Containers)

### Container 1 of 6

File Folder: 7a Item Number: 1 Page 1 of 5

AEH-12-PSEUDO-04

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

| PRINTED NAME | [redacted]RE | INITIALS | DATE |
|---|---|---|---|
| Berry L. Weber | [redacted] | Blw | [illegible] |
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"This Use and Maintenance Log Book has been inspected and found [redacted]liance with SOP GEN 009.

Inspected and sealed on [redacted] ."

Date Quality Assurance Unit

Page 2 of 5

ORIGINAL

SOP No. GEN 012.3
Page 5 of 7

Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401 SDP

Circle one: Test Article Control Article

Trade Name of Test Chemical (Synonyms) PF-CL 145A; Zequanox

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator

Date Received 07 AUG 12 KW Date Opened 17 AUG 12 KW Expiration Date 21 JUN 2013 (5 years unless otherwise stated)

Test Chemical Lot Number ~~401P121642~~ 401P121636 Purity of Chemical 52%

① Mixed upon receipt

Amount of Test Chemical Available or Received (if known) 500g

Initial Mass (with cover on) of Test Chemical and Container 541.45g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes X No __

Additional Comments about the Test Chemical:

① Test chemical was mixed by manufacturer and shipped to us in containers KW 09 AUG 12

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location __________ Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 09 AUG 12

Page 3 of 5

ORIGINAL

SOP No. GEN 012.3
Page 6 of 7

Form GEN 012.3b
Page 1 of 1

TEST CHEMICAL USE LOG

Test Chemical: MBI-401 SDP | Manufacturer: Marrone Bio Innovations | Lot or Batch #: 46IP121636, 46IP121642 Mixed

| A<br>Initial mass of test chemical & container with cap/ lid on<br>(g) | B<br>Amount removed<br>(g) | C<br>Mass of test chemical & container after removal, with cap/lid on<br>(g) | D<br>Study Number | E<br>Purpose and Other Comments | F<br>Date | G<br>Initials |
|---|---|---|---|---|---|---|
| *1 No archive sample needed as this | * | * | is not a regulated study | *Archived Sample | 09AUG12 | Kw |
| 541.40 | 2.0004 | 539.44 | AEH-12-PSEUDO-04 | Analytical Stock #1 | 12AUG12 | Kw |
| 539.44 | 2.0035 | 537.40 | ↑ | Analytical Stock #2 | ↑ | ↑ |
| 537.40 | 35.00 | 502.42 | | Stock #1 for Lake Carlos | | |
| 502.42 | 35.03 | 467.36 | | Stock #2 for Lake Carlos | | |
| 467.36 | 35.01 | 432.32 | | Stock #3 for Lake Carlos | | |
| 432.32 | 70.01 | 362.30 | | Stock #4 for Lake Carlos | | |
| 362.30 | 70.00 | 292.26 | | Stock #5 for Lake Carlos | | |
| 292.26 | 70.04 | 222.21 | ↓ | Stock #6 for Lake Carlos | ↓ | ↓ |
| 222.21 | 75.01 | 147.18 | AEH-12-PSEUDO-04 | Stock #7 for Lake Carlos | 12AUG12 | Kw |

* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

Page 4 of 5

ORIGINAL

SOP No. GEN 012.3
Page 7 of 7

Form GEN 012.3c
Page __ of __

TEST CHEMICAL USE LOG

Test Chemical: MBI-401 SDP
Manufacturer: Marrone Bio Innovations
Lot or Batch #: 401P121163C 401P121164C Mixed

| A<br>Initial mass of test chemical & container with cap/ lid on<br>(g) | B<br>Amount removed<br>(g) | C<br>Mass of test chemical & container after removal, with cap/lid on<br>(g) | D<br>Study Number | E<br>Purpose and Other Comments | F<br>Date | G<br>Initials |
|---|---|---|---|---|---|---|
| 147.18 | 6.83 | 140.32 | AEH-12-PSEUDO-04 | To NISM for post-test verification | 8 JAN 13 | [illegible] |
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A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

File Folder: 7a
Item Number: 1
Page 5 of 5

AEH-12-PSEUDO-04

# *CHEMICAL LOG BOOK*

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[redacted] Sealed Aug 9, 2012

## *MBI-401 SDP*

***Marrone Bio Innovations***
***Davis, California***

***LOT NUMBERS:***
**401P12163C**
**and**
**401P12164C**
**(Received Mixed in Containers)**

***Container 2 of 6***

File Folder: 7a  Item Number: 2  **Page** 1 **of** 4

## SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

| PRINTED NAME | SIGNATURE | INITIALS | DATE |
|---|---|---|---|
| Kerry L. Weber | [redacted] | KLW | 09/14/12 |
| JAMES A Luoma | [redacted] | JAL | 9/13/12 |

"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on August 9, 2012 (Date) by [redacted] Quality Assurance Unit."

Page 2 of 4

ORIGINAL



Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401 SDP

Circle one: Test Article Control Article

Trade Name of Test Chemical (Synonyms) Pf-CL 145A; Zequanox

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator

Date Received 07 AUG 12 [illegible] Date Opened 10 AUG 12 [illegible] Expiration Date 21 JUL 2013
(5 years unless otherwise stated)

Test Chemical Lot Number 401P12165C-① 401P12164C Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~500g

Initial Mass (with cover on) of Test Chemical and Container 556.52g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes X No __

Additional Comments about the Test Chemical:

① Received mixed from manufacturer [illegible] 09AUG12

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location ________ Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 09 AUG 12

Page 3 of 4

ORIGINAL



TEST CHEMICAL USE LOG

Test Chemical: MBI-401 SDP | Manufacturer: Marrone Bio Innovations | Lot or Batch #: 401P121636 401P121646 Mixed

| A<br>Initial mass of test chemical & container with cap/ lid on<br>(g) | B<br>Amount removed<br>(g) | C<br>Mass of test chemical & container after removal, with cap/lid on<br>(g) | D<br>Study Number | E<br>Purpose and Other Comments | F<br>Date | G<br>Initials |
|---|---|---|---|---|---|---|
| *1 No test | * chemical placed | * in archive | | *Archived Sample | 09AUG12 | KW |
| 556.55 | 150.05 | 406.44 | AEH-12-PSEUDO-04 | Stock #8 for Lake Co. lots | 12AUG12 | KW |
| 406.35 | 2.0009 | 404.38 | AEH12-PSEUDO-04 | Analytical #1 for Lake Pepin | 27AUG12 | KW |
| 404.38 | 2.00096 | 402.35 | | Analytical #2 for Lake Pepin | | |
| 402.35 | 35.05 | 362.23 | | Stock #1 for Lake Pepin ① ② | | |
| 362.23 | 35.03 | 327.19 | | Stock #2 for Lake Pepin ② | | |
| 327.19 | 35.00 | 292.20 | | Stock #3 for Lake Pepin ② | | |
| 292.20 | 70.05 | 222.12 | | Stock #4 for Lake Pepin ② | | |
| 222.12 | 70.04 | 151.95 | | Stock #5 for Lake Pepin ② | | |
| 151.95 | 70.07 | 81.85 | AEH-12-PSEUDO-04 | Stock #6 for Lake Pepin ② | 27AUG12 | KW |

* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

① Spilled ~5g while weighing out. KW 27AUG12

② Lake Pepin samples not used as trial was cancelled, retained and used for study #AEH-13-MBICKRDA-01 methods development 9/3/13 JDW

AEH-12-PSEUDO-04

File Folder: 7a

Item Number: 2

Page 4 of 4

AEH-12-PSEUDO-04

# CHEMICAL LOG BOOK

T OF THE INTERIOR
TY SEAL
TAMPER!
Aug. 7, 2012

## MBI-401 SDP

## Marrone Bio Innovations
## Davis, California

## LOT NUMBERS:
**401P12163C**
**and**
**401P12164C**
**(Received Mixed in Containers)**

## Container 3 of 6

File Folder: 7a    Item Number: 3    Page 1 of 4

AEH-12-PSEUDO-04

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

| PRINTED NAME | SIGNATURE | INITIALS | DATE |
|---|---|---|---|
| Kerry L. Weber | [redacted] | KLW | 09 AUG 10 |
| James A. Luoma | | JAL | 9/13/13 |
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"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on August 7, 2009 by [redacted] ."
Date Quality Assurance Unit

Page 2 of 4

ORIGINAL

AEH-12-PSEUDO-04
SOP No. GEN 012.3
Page 5 of 7

Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401 SDP

Circle one: Test Article Control Article

Trade Name of Test Chemical (Synonyms) Pf-CL145A; Zequanox

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator

Date Received 07AUG12 KW Date Opened 27 AUG 2012 KW Expiration Date 21 Jun 2013 (5 years unless otherwise stated)

Test Chemical Lot Number 401P121636 ~~401P121646~~ Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~520g

Initial Mass (with cover on) of Test Chemical and Container 536.87g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes X No __

Additional Comments about the Test Chemical:

①Received mixed from manufacturer KW 09AUG12

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location __________ Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signatur[redacted] Date 09AUG12

Page 3 of 4

ORIGINAL

SOP No. GEN 012.3
Page 6 of 7

Form GEN 012.3b
Page 1 of 1

# TEST CHEMICAL USE LOG

| Test Chemical: MBI-401 SDP | | | Manufacturer: Marrone Bio Innovations | Lot or Batch #: 401P121163C 401P121164C Mixed | | |
|---|---|---|---|---|---|---|
| A | B | C | D | E | F | G |
| Initial mass of test chemical & container with cap/ lid on (g) | Amount removed (g) | Mass of test chemical & container after removal, with cap/lid on (g) | Study Number | Purpose and Other Comments | Date | Initials |
| *1 No test chemical placed | * in archive | * | | *Archived Sample | 08AUG12 | Kw |
| 536.86 | 60.08 | 476.77 | AEH-12-PSEUDO-04 | Stock #7 for Lake Pepin ① | 27 AUG 12 | Kw |
| 476.77 | 120.07 | 356.65 | | Stock #8 for Lake Pepin ① | | |
| 356.65 | 2.00072 | 354.60 | | Analytical #1 for Shawano | | |
| 354.60 | 2.00029 | 352.59 | | Analytical #2 for Shawano | | |
| 352.59 | 35.10 | 317.49 | | Stock #1 for Shawano | | |
| 317.49 | 35.06 | 282.43 | | Stock #2 for Shawano | | |
| 282.43 | 35.07 | 247.33 | | Stock #3 for Shawano | | |
| 247.33 | 70.02 | 177.29 | | Stock #4 for Shawano | | |
| 177.29 | 70.04 | 107.25 | AEH-12-PSEUDO-04 | Stock #5 for Shawano | 27Aug12 | Kw |

* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
I = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).
① Stocks not used in Lake Pepin as trial was cancelled - retained and used for study #AEH-13-MBICRADA-01 methods development 9/13/13 JAL

AEH-12-PSEUDO-04

File Folder: 7a

Item Number: 3

Page 4 of 4

AEH-12-PSEUDO-04

# *CHEMICAL LOG BOOK*

## *MBI-401 SDP*

*Marrone Bio Innovations*
*Davis, California*

OF THE INTERIOR
Y SEAL
TAMPER!
Aug 7, 2013

*LOT NUMBERS:*
**401P12163C**
**and**
**401P12164C**
**(Received Mixed in Containers)**

*Container 4 of 6*

File Folder: 7a Item Number: 4 Page 1 of 4

SIGNATURE PAGE

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

| PRINTED NAME | SIGNATURE | INITIALS | DATE |
|---|---|---|---|
| Kerry Linebar | [redacted] | Kw | 09AUG12 |
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"This Use and Maintenance Log Book has been inspected [redacted] SOP GEN 009.

Inspected and sealed on August 9, 2012 by [redacted]."

Date  Quality Assurance Unit

Page 2 of 4

Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401 SDP

Circle one: Test Article  Control Article

Trade Name of Test Chemical (Synonyms) Pf-CL 145A; Zequanox

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator

Date Received 07AUG12 VW Date Opened 27 AUG 2012 VW Expiration Date 21 JUN 2013 (5 years unless otherwise stated)

Test Chemical Lot Number 4-31-12 R-36 ① Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~520g

Initial Mass (with cover on) of Test Chemical and Container 540.24g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes X No __

Additional Comments about the Test Chemical:

① Received mixed from manufacturer. VW 09AUG12

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location ________ Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 09AUG12

Page 3 of 4

ORIGINAL

SOP No. GEN 012.3
Page 6 of 7

Form GEN 012.3b
Page 1 of 1

TEST CHEMICAL USE LOG

| Test Chemical: MBI-401 SDP | | | Manufacturer: Marrone Bio Innovations | Lot or Batch #: 401P121636 401P121646 Mixed | | |
|---|---|---|---|---|---|---|
| A | B | C | D | E | F | G |
| Initial mass of test chemical & container with cap/ lid on (g) | Amount removed (g) | Mass of test chemical & container after removal, with cap/lid on (g) | Study Number | Purpose and Other Comments | Date | Initials |
| *1 No test chemical placed | * | * in archive | | *Archived Sample | 09AUG12 | KW |
| 540.40 | 70.02 | 470.36 | AEH-12-PSEUDO-04 | Stock #6 for Shawano | 27AUG12 | KW |
| 470.36 | 60.06 | 410.25 | ↕ | Stock #7 for Shawano | ↕ | ↕ |
| 410.25 | 120.07 | 290.10 | AEH-12-PSEUDO-04 | Stock #8 for Shawano | 27AUG12 | KW |
| 190.30 | 100.05 | 190.24 | AEH-13-~~MBICRADA~~-01 ① | ① wrote over: MBICRADA Injection procedure KW 05SEP13 | 05SEP13 | KW |
| 190.31 | 100.03 | 90.26 | ~~N/A~~ ② | ② Incorrect: Study number is: AEH-13-MBICRADA-01 KW 06SEP13 | 06SEP13 | KW |
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* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

AEH-12-PSEUDO-04

File Folder: 7a

Item Number: 4

Page 4 of 4

AEH-12-PSEUDO-04

# *CHEMICAL LOG BOOK*

## *MBI-401 SDP*

## *Marrone Bio Innovations*
## *Davis, California*

ealed August 9, 2012

## *LOT NUMBERS:*
## 401P12163C
## and
## 401P12164C
## (Received Mixed in Containers)

## *Container 6 of 6*

File Folder: 7a Item Number: 5 **Page** 1 **of** 4

SIGNATURE PAGE AEH-12-PSEUDO-04

All personnel making an entry in this log must fill out the form below in accordance with SOP GEN 009.

| PRINTED NAME | SIGNATURE | INITIALS | DATE |
|---|---|---|---|
| Kerry Liwelx | [redacted] | Klw | 08/16/13 |
| James A. LaoMA | | JA | 7/11/13 |
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"This Use and Maintenance Log Book has been inspected and found to be in compliance with SOP GEN 009.

Inspected and sealed on ____________ by ____________."
Date Quality Assurance Unit

Page 2 of 4

ORIGINAL



Form GEN 012.3a
Page 1 of 1

TEST CHEMICAL DATA FORM

Test Chemical (Chemical Name) MBI-401 SDP

Circle one: Test Article Control Article

Trade Name of Test Chemical (Synonyms) PF-CL145A; Zequanox

Source of Test Chemical (Manufacturer) Marrone Bio Innovations

Storage Location Refrigerator

Date Received 07AUG12 KW Date Opened 14 AUG 2012 KW Expiration Date 21 JAN 13 (5 years unless otherwise stated)

Test Chemical Lot Number ~~401P121642~~ 401P121634 ① Purity of Chemical 50%

Amount of Test Chemical Available or Received (if known) ~2500g

Initial Mass (with cover on) of Test Chemical and Container 539.20g

Characterization of Test Chemical: Color Tan
Physical State: liquid __ solid X
Solid Form: powder X crystal __ pellet __

Chemical Abstract Service Number N/A

Manufacturer Certificate of Analysis Yes X No __

Additional Comments about the Test Chemical:

① Received mixed from manufacturer. KW 09AUG12

Sample Placed in Archives: Yes __ No X (Entries should also be made on Form GEN 012.b)

Archive Location ____________ Material Safety Data Sheet Available: Yes X No __

Signature of Study Director or designee initiating Test Chemical Use Log and date:

Signature [redacted] Date 09AUG12

Page 3 of 4

ORIGINAL



Form GEN 012.3b
Page 1 of 1

# TEST CHEMICAL USE LOG

| Test Chemical: MBI-401 SDP | | Manufacturer: Marrone Bio Innovations | | Lot or Batch #: 401P12163C 401P12164C Mixed | | |
|---|---|---|---|---|---|---|
| A | B | C | D | E | F | G |
| Initial mass of test chemical & container with cap/ lid on (g) | Amount removed (g) | Mass of test chemical & container after removal, with cap/lid on (g) | Study Number | Purpose and Other Comments | Date | Initials |
| *1 No test chemical placed | * | * in archive | | *Archived Sample | 09AUG12 | Kw |
| 539.1 | 1.0 | 538.0 | AEH-12-PSEUDO-04 | Sample to make stock for lab testing for disposal | 14AUG12 | Kw |
| 538.51 | 312.10 | 226.41 | AEH-13-MBICAADA-01 | injection methods development | 9/1/13 | JR |
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* = The first entry should be the test chemical sample placed in the Chemical Archives. Follow GEN 011.
1 = The initial mass is also entered on Form GEN 012.a.
A. The mass of the test chemical and its container will be determined using a balance that has been verified to be accurate (SOP GEN 013).
B. The test chemical removed from the container will be placed into a tared vessel.
C. After the test chemical has been removed from the container, determine the mass of the container and its contents (with cap/lid on).

AEH-12-PSEUDO-04

File Folder: 7a
Item Number: 5
Page 4 of 4

# Appendix 5. Test Animal Information

ORIGINAL

Form GEN 134.4a
Page 1 of 1

APPROVAL FOR HOUSING AND CARE OF TEST ANIMALS DURING EXPERIMENTS

This protocol has been examined by the Animal Care and Use Committee for consistency with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and with rules governing the use of test animals at the Upper Midwest environmental Sciences Center, La Crosse.

We, the undersigned, find this protocol to be acceptable as it is represented.

Protocol Number: AE 14-12-PSEUDO-04

Principal Investigator: James A. Luoma

Study Director: ______

Protocol Title: Efficacy of Pseudomonas fluorescens (Pf-CL145A) SDP for controlling settled zebra mussels on artificial substrates

[redacted] Chair — 5-1-12
Signature, Title — Date

No additional ACU review needed because no vertebrates are part of this study
Signature, Title — Date

______
Signature, Title — Date

______
Signature, Title — Date

* File the original signed copy of this form in the study file for each study and the Chair, Animal Care and Use Committee gets a copy.

File Folder: 6

Item Number: 1

Page 1 of 1

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 30) | Created...... | 28-Oct-13 | KLW |
| Data Source: File Folder: 15 | Revised...... | 12-Feb-14 | TJS |
| Forms: "Zebra Mussel Lengths" | Reviewed... | 12 FEB 14 | TJS |
| | Certified... | 2/14/14 | JAL |
| File Name: See filenames as stated below | | | |

**Zebra Mussel Lengths - Lake Carlos (Whole Water Column)**

Test Article: MBI 401 SDP (*Pseudomonas fluorescens Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

Overall Data Summary:

| | 6 h | | | 9 h | | | 12 h | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 mg/L | 50 mg/L | 100 mg/L | 0 mg/L | 50 mg/L | 100 mg/L | 0 mg/L | 50 mg/L | 100 mg/L |
| Mean (mm) | 11.51 | 12.41 | 10.66 | 11.26 | 11.64 | 11.37 | 11.79 | 11.33 | 10.67 |
| (STD) | (2.41) | (2.64) | (2.85) | (2.44) | (2.61) | (2.74) | (2.76) | (2.82) | (2.48) |
| Minimum | 6.15 | 6.94 | 6.00 | 6.43 | 6.70 | 6.43 | 6.19 | 6.04 | 6.02 |
| Maximum | 19.47 | 17.79 | 18.33 | 17.17 | 18.10 | 18.19 | 19.03 | 24.27 | 17.78 |
| Grand Mean | | 11.53 | | | 11.42 | | | 11.26 | |
| (STD) | | (0.71) | | | (0.16) | | | (0.46) | |

Data Explanation:
After survival analysis, one tray from each treatment level (0, 50 and 100 mg/L) at each exposure termination time point (6, 9 and 12 h) was retained for mussel length analysis. All animals were measured for length. All lengths were reported except for those from mussels with broken shells or those < 6 mm.

6 h Mussel Length Data
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Lengths (Whole Tank).xlsx]6h Length Data

9 h Mussel Length Data
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Lengths (Whole Tank).xlsx]9h Length Data

12 h Mussel Length Data
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Lengths (Whole Tank).xlsx]12h Length Data

Data anomalies and deviations:
NONE

File Folder: 15

Item Number 1
Page 1 of 4

Study Number: AEH-12-PSEUDC-04
Electronic Lab Notebook (pages 50)
Data Source: File Folder: 15
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP [Pf-CL145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

# 6 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 mg/L | W5C1 | 183 | 6.15 | 6.36 | 6.51 | 6.62 | 6.67 | 6.70 | 7.08 | 7.30 | 7.56 | 7.83 | 7.90 | 7.96 | 7.97 | 7.99 | 8.10 | 8.25 | 8.27 | 8.33 | 8.39 | 8.50 | 11.51 (2.41) |
| | | | 8.58 | 8.58 | 8.62 | 8.63 | 8.67 | 8.74 | 8.78 | 8.94 | 8.95 | 8.96 | 8.97 | 9.02 | 9.04 | 9.06 | 9.12 | 9.13 | 9.15 | 9.29 | 9.30 | 9.42 | |
| | | | 9.43 | 9.53 | 9.66 | 9.69 | 9.81 | 9.82 | 9.85 | 9.86 | 9.94 | 10.10 | 10.11 | 10.16 | 10.18 | 10.18 | 10.18 | 10.23 | 10.28 | 10.29 | 10.37 | 10.40 | |
| | | | 10.48 | 10.54 | 10.55 | 10.57 | 10.73 | 10.74 | 10.75 | 10.89 | 10.91 | 10.94 | 10.94 | 11.07 | 11.16 | 11.16 | 11.18 | 11.19 | 11.23 | 11.24 | 11.24 | 11.28 | |
| | | | 11.29 | 11.32 | 11.33 | 11.35 | 11.39 | 11.42 | 11.50 | 11.50 | 11.52 | 11.53 | 11.56 | 11.58 | 11.60 | 11.60 | 11.60 | 11.63 | 11.64 | 11.71 | 11.75 | 11.80 | |
| | | | 11.81 | 11.81 | 11.82 | 11.83 | 11.85 | 11.85 | 11.86 | 11.95 | 11.98 | 12.00 | 12.03 | 12.09 | 12.11 | 12.11 | 12.13 | 12.30 | 12.32 | 12.44 | 12.47 | 12.48 | |
| | | | 12.49 | 12.52 | 12.53 | 12.53 | 12.53 | 12.66 | 12.70 | 12.71 | 12.79 | 12.81 | 12.82 | 12.82 | 12.85 | 12.87 | 12.90 | 12.94 | 12.94 | 12.97 | 13.04 | 13.06 | |
| | | | 13.17 | 13.18 | 13.26 | 13.28 | 13.31 | 13.47 | 13.48 | 13.58 | 13.62 | 13.72 | 13.75 | 13.76 | 13.77 | 13.88 | 14.06 | 14.10 | 14.15 | 14.16 | 14.17 | 14.28 | |
| | | | 14.33 | 14.35 | 14.42 | 14.49 | 14.49 | 14.50 | 14.51 | 14.73 | 14.87 | 15.08 | 15.21 | 15.37 | 15.44 | 15.50 | 15.59 | 15.63 | 15.82 | 16.02 | 16.14 | 16.22 | |
| | | | 16.48 | 18.63 | 19.47 | | | | | | | | | | | | | | | | | | |
| 50 mg/L | W1A3 | 152 | 6.94 | 7.22 | 7.45 | 7.48 | 7.50 | 7.70 | 7.87 | 8.03 | 8.04 | 8.15 | 8.15 | 8.28 | 8.28 | 8.66 | 8.77 | 8.78 | 8.89 | 8.92 | 8.98 | 9.10 | 12.41 (2.64) |
| | | | 9.12 | 9.14 | 9.33 | 9.36 | 9.37 | 9.49 | 9.56 | 9.70 | 9.85 | 9.99 | 10.00 | 10.02 | 10.13 | 10.14 | 10.17 | 10.19 | 10.28 | 10.28 | 10.49 | 10.52 | |
| | | | 10.58 | 10.61 | 10.66 | 10.81 | 10.88 | 10.88 | 10.88 | 10.91 | 10.91 | 10.91 | 10.96 | 11.10 | 11.21 | 11.26 | 11.27 | 11.38 | 11.44 | 11.50 | 11.54 | 11.63 | |
| | | | 11.68 | 11.89 | 11.93 | 12.05 | 12.09 | 12.17 | 12.24 | 12.29 | 12.32 | 12.36 | 12.37 | 12.42 | 12.47 | 12.50 | 12.56 | 12.60 | 12.62 | 12.64 | 12.68 | 12.76 | |
| | | | 12.78 | 12.81 | 12.81 | 12.92 | 12.99 | 13.19 | 13.19 | 13.23 | 13.43 | 13.44 | 13.45 | 13.48 | 13.53 | 13.55 | 13.58 | 13.65 | 13.69 | 13.73 | 13.80 | 13.80 | |
| | | | 13.84 | 13.86 | 13.87 | 13.90 | 13.91 | 13.98 | 13.98 | 14.03 | 14.04 | 14.11 | 14.13 | 14.13 | 14.14 | 14.17 | 14.18 | 14.20 | 14.25 | 14.26 | 14.30 | 14.36 | |
| | | | 14.38 | 14.44 | 14.45 | 14.45 | 14.59 | 14.60 | 14.61 | 14.62 | 14.80 | 14.82 | 14.89 | 14.91 | 14.98 | 15.07 | 15.31 | 15.49 | 15.56 | 15.60 | 16.19 | 16.21 | |
| | | | 16.36 | 16.99 | 17.17 | 17.21 | 17.31 | 17.42 | 17.65 | 17.65 | 17.70 | 17.71 | 17.79 | 17.79 | | | | | | | | | |
| 100 mg/L | W7B2 | 271 | 6.00 | 6.02 | 6.15 | 6.21 | 6.24 | 6.26 | 6.32 | 6.39 | 6.45 | 6.53 | 6.54 | 6.57 | 6.58 | 6.61 | 6.63 | 6.83 | 6.87 | 6.99 | 7.00 | 7.05 | 10.66 (2.85) |
| | | | 7.12 | 7.12 | 7.15 | 7.18 | 7.21 | 7.21 | 7.26 | 7.30 | 7.31 | 7.34 | 7.40 | 7.41 | 7.43 | 7.43 | 7.44 | 7.45 | 7.50 | 7.53 | 7.57 | 7.57 | |
| | | | 7.57 | 7.58 | 7.65 | 7.65 | 7.76 | 7.86 | 7.87 | 7.88 | 7.88 | 7.88 | 7.90 | 7.92 | 7.92 | 7.92 | 7.95 | 7.95 | 7.96 | 7.98 | 8.07 | 8.07 | |
| | | | 8.08 | 8.12 | 8.15 | 8.17 | 8.17 | 8.18 | 8.20 | 8.21 | 8.22 | 8.24 | 8.29 | 8.29 | 8.32 | 8.32 | 8.34 | 8.34 | 8.40 | 8.42 | 8.42 | 8.47 | |
| | | | 8.47 | 8.49 | 8.49 | 8.57 | 8.64 | 8.67 | 8.70 | 8.73 | 8.74 | 8.78 | 8.79 | 8.82 | 8.82 | 8.88 | 8.97 | 9.00 | 9.00 | 9.02 | 9.07 | 9.08 | |
| | | | 9.12 | 9.14 | 9.16 | 9.20 | 9.20 | 9.28 | 9.35 | 9.37 | 9.44 | 9.45 | 9.46 | 9.47 | 9.49 | 9.49 | 9.51 | 9.52 | 9.54 | 9.56 | 9.58 | 9.61 | |
| | | | 9.65 | 9.67 | 9.67 | 9.70 | 9.72 | 9.73 | 9.76 | 9.76 | 9.77 | 9.80 | 9.81 | 9.84 | 9.85 | 9.96 | 10.01 | 10.07 | 10.21 | 10.31 | 10.32 | 10.33 | |
| | | | 10.37 | 10.38 | 10.43 | 10.45 | 10.65 | 10.68 | 10.71 | 10.74 | 10.76 | 10.84 | 10.84 | 10.85 | 10.91 | 10.92 | 11.04 | 11.04 | 11.17 | 11.29 | 11.31 | 11.37 | |
| | | | 11.37 | 11.43 | 11.43 | 11.49 | 11.53 | 11.54 | 11.56 | 11.59 | 11.64 | 11.68 | 11.76 | 11.77 | 11.78 | 11.78 | 11.80 | 11.82 | 11.82 | 11.85 | 11.88 | 11.91 | |
| | | | 11.93 | 11.94 | 11.95 | 12.05 | 12.09 | 12.20 | 12.23 | 12.23 | 12.26 | 12.29 | 12.35 | 12.46 | 12.47 | 12.57 | 12.60 | 12.60 | 12.69 | 12.72 | 12.73 | 12.73 | |
| | | | 12.74 | 12.77 | 12.77 | 12.80 | 12.85 | 13.02 | 13.09 | 13.13 | 13.24 | 13.25 | 13.25 | 13.25 | 13.25 | 13.28 | 13.29 | 13.34 | 13.40 | 13.49 | 13.49 | 13.50 | |
| | | | 13.53 | 13.61 | 13.70 | 13.74 | 13.86 | 13.89 | 13.93 | 13.97 | 14.00 | 14.00 | 14.05 | 14.05 | 14.06 | 14.07 | 14.16 | 14.24 | 14.26 | 14.30 | 14.46 | 14.73 | |
| | | | 14.76 | 14.76 | 14.79 | 14.79 | 14.91 | 15.00 | 15.09 | 15.10 | 15.14 | 15.16 | 15.22 | 15.23 | 15.39 | 15.41 | 15.49 | 15.53 | 15.57 | 15.57 | 15.62 | 15.73 | |
| | | | 15.98 | 16.03 | 16.09 | 16.31 | 16.40 | 16.49 | 16.50 | 16.62 | 16.80 | 17.08 | 18.33 | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | 11.53 (0.71) |

Item Number 1
Page 2 of 4

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 33)
Data Source: File Folder: 15
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

# 9 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6.43 | 6.48 | 6.52 | 6.57 | 6.78 | 7.09 | 7.12 | 7.20 | 7.20 | 7.27 | 7.28 | 7.36 | 7.52 | 7.62 | 7.65 | 7.91 | 7.96 | 7.97 | 7.99 | 8.03 | |
| | | | 8.07 | 8.11 | 8.14 | 8.15 | 8.17 | 8.19 | 8.20 | 8.35 | 8.37 | 8.37 | 8.38 | 8.41 | 8.70 | 8.79 | 8.79 | 8.80 | 8.83 | 8.85 | 8.86 | 8.93 | |
| | | | 8.97 | 9.04 | 9.07 | 9.10 | 9.10 | 9.33 | 9.53 | 9.55 | 9.60 | 9.61 | 9.66 | 9.67 | 9.72 | 9.73 | 9.79 | 9.79 | 9.82 | 9.82 | 9.87 | 9.96 | |
| | | | 9.97 | 9.99 | 10.02 | 10.07 | 10.10 | 10.22 | 10.23 | 10.27 | 10.30 | 10.34 | 10.38 | 10.39 | 10.39 | 10.41 | 10.41 | 10.52 | 10.52 | 10.55 | 10.56 | 10.61 | 11.26 |
| 0 mg/L | W5B3 | 193 | 10.67 | 10.75 | 10.75 | 10.85 | 10.92 | 10.92 | 10.94 | 10.94 | 10.98 | 10.99 | 11.05 | 11.08 | 11.14 | 11.26 | 11.28 | 11.32 | 11.33 | 11.35 | 11.44 | 11.47 | (2.44) |
| | | | 11.47 | 11.51 | 11.55 | 11.57 | 11.57 | 11.58 | 11.58 | 11.59 | 11.59 | 11.62 | 11.65 | 11.67 | 11.69 | 11.69 | 11.73 | 11.81 | 11.82 | 11.90 | 11.98 | 11.98 | |
| | | | 12.01 | 12.01 | 12.05 | 12.17 | 12.19 | 12.23 | 12.25 | 12.29 | 12.30 | 12.31 | 12.40 | 12.42 | 12.43 | 12.46 | 12.46 | 12.55 | 12.57 | 12.58 | 12.61 | 12.64 | |
| | | | 12.67 | 12.68 | 12.73 | 12.74 | 12.80 | 12.84 | 12.98 | 13.07 | 13.11 | 13.12 | 13.21 | 13.24 | 13.37 | 13.38 | 13.45 | 13.54 | 13.58 | 13.66 | 13.73 | 13.78 | |
| | | | 13.79 | 13.87 | 13.91 | 13.91 | 13.99 | 14.12 | 14.35 | 14.46 | 14.50 | 14.57 | 14.59 | 14.62 | 14.74 | 14.77 | 14.80 | 14.82 | 14.86 | 14.87 | 14.87 | 14.94 | |
| | | | 15.06 | 15.34 | 15.45 | 15.48 | 15.60 | 15.67 | 15.75 | 15.91 | 15.96 | 15.98 | 16.02 | 16.49 | 17.17 | | | | | | | | |
| | | | 6.70 | 6.81 | 6.89 | 7.09 | 7.25 | 7.33 | 7.34 | 7.37 | 7.42 | 7.42 | 7.44 | 7.44 | 7.45 | 7.53 | 7.67 | 7.80 | 7.82 | 7.84 | 7.86 | 7.89 | |
| | | | 7.90 | 8.11 | 8.11 | 8.14 | 8.15 | 8.16 | 8.24 | 8.27 | 8.30 | 8.30 | 8.31 | 8.32 | 8.36 | 8.39 | 8.40 | 8.41 | 8.45 | 8.49 | 8.57 | 8.59 | |
| | | | 8.60 | 8.63 | 8.65 | 8.66 | 8.67 | 8.77 | 8.79 | 8.81 | 8.91 | 8.92 | 8.94 | 9.04 | 9.05 | 9.10 | 9.10 | 9.12 | 9.16 | 9.22 | 9.24 | 9.26 | |
| | | | 9.29 | 9.30 | 9.30 | 9.31 | 9.32 | 9.33 | 9.33 | 9.38 | 9.41 | 9.52 | 9.53 | 9.58 | 9.58 | 9.62 | 9.63 | 9.68 | 9.69 | 9.70 | 9.74 | 9.78 | |
| | | | 9.79 | 9.81 | 9.84 | 9.84 | 9.86 | 9.96 | 9.98 | 10.10 | 10.15 | 10.23 | 10.26 | 10.31 | 10.32 | 10.33 | 10.40 | 10.42 | 10.50 | 10.53 | 10.54 | 10.60 | 11.64 |
| | | | 10.63 | 10.66 | 10.68 | 10.68 | 10.70 | 10.76 | 10.79 | 10.88 | 10.91 | 10.94 | 10.98 | 11.04 | 11.08 | 11.11 | 11.13 | 11.15 | 11.15 | 11.16 | 11.20 | 11.24 | (2.61) |
| 50 mg/L | W4B3 | 264 | 11.29 | 11.30 | 11.30 | 11.36 | 11.39 | 11.39 | 11.51 | 11.55 | 11.55 | 11.56 | 11.57 | 11.68 | 11.71 | 11.77 | 11.77 | 11.78 | 11.79 | 11.79 | 11.88 | 11.89 | |
| | | | 11.91 | 11.95 | 12.11 | 12.11 | 12.13 | 12.15 | 12.17 | 12.17 | 12.23 | 12.24 | 12.31 | 12.32 | 12.36 | 12.39 | 12.44 | 12.47 | 12.49 | 12.54 | 12.57 | 12.59 | |
| | | | 12.65 | 12.66 | 12.70 | 12.71 | 12.79 | 12.80 | 12.84 | 12.84 | 12.87 | 12.87 | 12.96 | 12.98 | 13.02 | 13.02 | 13.02 | 13.04 | 13.05 | 13.11 | 13.12 | 13.13 | |
| | | | 13.14 | 13.16 | 13.19 | 13.24 | 13.30 | 13.34 | 13.34 | 13.36 | 13.45 | 13.47 | 13.55 | 13.56 | 13.60 | 13.61 | 13.64 | 13.64 | 13.65 | 13.65 | 13.66 | 13.67 | |
| | | | 13.57 | 13.74 | 13.76 | 13.78 | 13.85 | 13.86 | 13.91 | 13.92 | 14.07 | 14.11 | 14.11 | 14.13 | 14.14 | 14.17 | 14.17 | 14.19 | 14.22 | 14.24 | 14.24 | 14.27 | |
| | | | 14.27 | 14.30 | 14.39 | 14.40 | 14.43 | 14.44 | 14.45 | 14.47 | 14.52 | 14.55 | 14.55 | 14.62 | 14.64 | 14.69 | 14.77 | 14.80 | 14.83 | 14.89 | 14.92 | 15.13 | |
| | | | 15.18 | 15.23 | 15.25 | 15.26 | 15.36 | 15.45 | 15.47 | 15.52 | 15.80 | 15.83 | 15.84 | 15.99 | 16.03 | 16.08 | 16.21 | 16.35 | 16.64 | 16.68 | 16.72 | 16.93 | |
| | | | 16.94 | 17.84 | 18.09 | 18.10 | | | | | | | | | | | | | | | | | |
| | | | 6.43 | 6.57 | 6.71 | 6.78 | 6.85 | 7.05 | 7.16 | 7.16 | 7.23 | 7.34 | 7.53 | 7.55 | 7.67 | 7.68 | 7.72 | 7.74 | 7.77 | 7.80 | 7.88 | 7.93 | |
| | | | 7.95 | 8.02 | 8.02 | 8.05 | 8.11 | 8.13 | 8.14 | 8.32 | 8.32 | 8.41 | 8.47 | 8.48 | 8.54 | 8.56 | 8.59 | 8.74 | 8.77 | 8.77 | 8.82 | 8.87 | |
| | | | 8.89 | 8.91 | 8.92 | 8.95 | 8.95 | 9.13 | 9.15 | 9.35 | 9.41 | 9.43 | 9.51 | 9.55 | 9.59 | 9.60 | 9.61 | 9.65 | 9.70 | 9.73 | 9.75 | 9.79 | |
| | | | 9.79 | 9.79 | 9.90 | 9.91 | 9.93 | 9.99 | 10.12 | 10.13 | 10.14 | 10.16 | 10.17 | 10.20 | 10.21 | 10.22 | 10.26 | 10.31 | 10.33 | 10.39 | 10.47 | 10.53 | 11.37 |
| 100 mg/L | W6B2 | 189 | 10.61 | 10.67 | 10.76 | 10.84 | 10.84 | 10.86 | 10.86 | 10.88 | 10.92 | 10.93 | 10.95 | 10.96 | 10.96 | 10.97 | 10.98 | 10.99 | 11.07 | 11.11 | 11.16 | 11.21 | (2.74) |
| | | | 11.23 | 11.23 | 11.28 | 11.30 | 11.34 | 11.34 | 11.39 | 11.46 | 11.49 | 11.51 | 11.54 | 11.55 | 11.56 | 11.59 | 11.64 | 11.80 | 11.87 | 12.10 | 12.11 | 12.15 | |
| | | | 12.19 | 12.21 | 12.24 | 12.25 | 12.30 | 12.35 | 12.39 | 12.55 | 12.62 | 12.75 | 12.89 | 12.93 | 13.05 | 13.08 | 13.21 | 13.21 | 13.26 | 13.36 | 13.36 | 13.39 | |
| | | | 13.48 | 13.52 | 13.53 | 13.62 | 13.63 | 13.66 | 13.69 | 13.74 | 13.75 | 13.78 | 13.91 | 13.96 | 14.15 | 14.29 | 14.30 | 14.46 | 14.48 | 14.50 | 14.53 | 14.55 | |
| | | | 14.58 | 14.60 | 14.73 | 14.83 | 14.87 | 14.87 | 14.90 | 15.03 | 15.05 | 15.08 | 15.10 | 15.16 | 15.27 | 15.35 | 15.49 | 15.59 | 15.60 | 16.05 | 16.11 | 16.16 | |
| | | | 16.19 | 16.53 | 16.70 | 16.71 | 17.12 | 17.75 | 17.83 | 17.84 | 18.19 | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | 11.42 (0.16) |

Item Number 1
Page 3 of 4

Study Number: AE-I-12-PSEUDO 14
Electronic Lab Notebook (pages 30)
Data Source: File Folder: 15
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP (*Pf*-CL145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

# 12 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6.19 | 6.27 | 6.38 | 6.38 | 6.48 | 6.51 | 6.57 | 6.67 | 6.77 | 6.84 | 6.98 | 7.33 | 7.36 | 7.42 | 7.46 | 7.50 | 7.59 | 7.83 | 7.87 | 7.91 | |
| | | | 8.10 | 8.12 | 8.15 | 8.19 | 8.21 | 8.23 | 8.24 | 8.27 | 8.36 | 8.42 | 8.44 | 8.78 | 9.08 | 9.18 | 9.21 | 9.43 | 9.48 | 9.52 | 9.53 | 9.58 | |
| | | | 9.62 | 9.65 | 9.66 | 9.78 | 9.85 | 9.90 | 9.96 | 9.99 | 10.18 | 10.23 | 10.26 | 10.27 | 10.27 | 10.30 | 10.38 | 10.39 | 10.45 | 10.45 | 10.51 | 10.52 | |
| | | | 10.52 | 10.56 | 10.58 | 10.58 | 10.60 | 10.65 | 10.66 | 10.66 | 10.67 | 10.73 | 10.75 | 10.81 | 10.89 | 10.96 | 11.05 | 11.15 | 11.26 | 11.30 | 11.40 | 11.41 | 11.79 |
| 0 mg/L | W3A2 | 188 | 11.42 | 11.51 | 11.51 | 11.63 | 11.67 | 11.68 | 11.68 | 11.75 | 11.75 | 11.78 | 11.78 | 11.81 | 11.81 | 11.91 | 11.94 | 11.95 | 11.97 | 12.00 | 12.03 | 12.08 | (2.76) |
| | | | 12.16 | 12.17 | 12.23 | 12.26 | 12.27 | 12.30 | 12.34 | 12.36 | 12.42 | 12.48 | 12.49 | 17.50 | 12.54 | 12.55 | 12.57 | 12.61 | 12.67 | 12.68 | 12.71 | 12.73 | |
| | | | 12.73 | 12.77 | 12.86 | 13.04 | 13.04 | 13.07 | 13.15 | 13.16 | 13.24 | 13.26 | 13.27 | 13.27 | 13.33 | 13.34 | 13.37 | 13.46 | 13.50 | 13.53 | 13.54 | 13.65 | |
| | | | 13.69 | 13.79 | 13.82 | 13.86 | 13.90 | 14.03 | 14.05 | 14.12 | 14.13 | 14.24 | 14.35 | 14.37 | 14.41 | 14.42 | 14.57 | 14.57 | 14.61 | 14.62 | 14.69 | 14.71 | |
| | | | 14.72 | 14.75 | 14.76 | 14.85 | 14.87 | 14.93 | 15.07 | 15.09 | 15.26 | 15.32 | 15.35 | 15.41 | 15.43 | 15.55 | 15.65 | 15.78 | 15.83 | 15.83 | 16.31 | 16.58 | |
| | | | 16.71 | 16.78 | 16.83 | 17.28 | 17.30 | 17.41 | 18.11 | 19.08 | | | | | | | | | | | | | |
| | | | 6.04 | 6.06 | 6.15 | 6.21 | 6.31 | 6.32 | 6.34 | 6.43 | 6.43 | 6.60 | 6.64 | 6.69 | 6.71 | 6.77 | 6.80 | 6.84 | 6.91 | 6.92 | 6.97 | 7.04 | |
| | | | 7.08 | 7.16 | 7.25 | 7.33 | 7.51 | 7.52 | 7.66 | 7.70 | 7.71 | 7.82 | 7.85 | 7.92 | 7.99 | 8.00 | 8.00 | 8.03 | 8.21 | 8.25 | 8.27 | 8.29 | |
| | | | 8.35 | 8.47 | 8.48 | 8.51 | 8.54 | 8.55 | 8.73 | 8.76 | 8.79 | 8.80 | 8.82 | 8.83 | 8.85 | 8.89 | 8.91 | 8.91 | 8.95 | 9.02 | 9.02 | 9.06 | |
| | | | 9.08 | 9.19 | 9.25 | 9.32 | 9.34 | 9.35 | 9.37 | 9.41 | 9.42 | 9.43 | 9.45 | 9.45 | 9.48 | 9.51 | 9.55 | 9.60 | 9.64 | 9.65 | 9.66 | 9.68 | |
| | | | 9.68 | 9.69 | 9.72 | 9.73 | 9.74 | 9.75 | 9.78 | 9.79 | 9.84 | 9.88 | 9.89 | 9.90 | 9.93 | 9.94 | 9.96 | 9.97 | 9.99 | 10.03 | 10.07 | 10.07 | |
| | | | 10.17 | 10.19 | 10.19 | 10.25 | 10.31 | 10.33 | 10.33 | 10.33 | 10.35 | 10.37 | 10.38 | 10.45 | 10.46 | 10.46 | 10.46 | 10.50 | 10.54 | 10.57 | 10.62 | 10.63 | |
| 50 mg/L | W1C3 | 267 | 10.70 | 10.77 | 10.77 | 10.85 | 10.86 | 10.91 | 10.93 | 10.93 | 10.94 | 10.95 | 10.95 | 10.98 | 11.00 | 11.03 | 11.06 | 11.08 | 11.11 | 11.19 | 11.19 | 11.24 | 11.33 |
| | | | 11.30 | 11.30 | 11.39 | 11.43 | 11.48 | 11.62 | 11.66 | 11.72 | 11.74 | 11.84 | 11.89 | 11.91 | 11.94 | 11.94 | 11.96 | 11.99 | 11.99 | 12.00 | 12.04 | 12.12 | (2.82) |
| | | | 12.23 | 12.27 | 12.28 | 12.29 | 12.33 | 12.33 | 12.35 | 12.41 | 12.43 | 12.45 | 12.48 | 12.49 | 12.50 | 12.51 | 12.60 | 12.61 | 12.63 | 12.64 | 12.67 | 12.63 | |
| | | | 12.81 | 12.82 | 12.85 | 12.87 | 12.87 | 12.88 | 12.92 | 12.98 | 13.06 | 13.08 | 13.09 | 13.23 | 13.26 | 13.25 | 13.31 | 13.33 | 13.34 | 13.37 | 13.42 | 13.42 | |
| | | | 13.43 | 13.43 | 13.44 | 13.45 | 13.49 | 13.59 | 13.67 | 13.78 | 13.80 | 13.83 | 13.86 | 13.86 | 13.87 | 13.87 | 13.88 | 13.89 | 13.90 | 13.90 | 13.92 | 13.93 | |
| | | | 14.02 | 14.04 | 14.08 | 14.15 | 14.22 | 14.25 | 14.32 | 14.37 | 14.41 | 14.43 | 14.45 | 14.46 | 14.48 | 14.55 | 14.56 | 14.62 | 14.77 | 14.81 | 14.86 | 14.37 | |
| | | | 14.89 | 14.94 | 14.96 | 15.08 | 15.03 | 15.12 | 15.22 | 15.23 | 15.24 | 15.35 | 15.39 | 15.57 | 15.60 | 15.74 | 15.98 | 16.11 | 16.46 | 16.47 | 16.51 | 16.54 | |
| | | | 16.64 | 16.65 | 17.01 | 17.35 | 17.54 | 17.75 | 24.27 | | | | | | | | | | | | | | |
| | | | 6.02 | 6.02 | 6.03 | 6.21 | 6.24 | 6.29 | 6.32 | 6.35 | 6.51 | 6.73 | 6.77 | 6.90 | 6.90 | 6.92 | 6.92 | 6.96 | 7.00 | 7.05 | 7.07 | 7.25 | |
| | | | 7.33 | 7.41 | 7.45 | 7.47 | 7.55 | 7.62 | 7.65 | 7.66 | 7.67 | 7.69 | 7.75 | 7.76 | 7.80 | 7.85 | 7.86 | 7.86 | 7.87 | 7.96 | 7.97 | 8.02 | |
| | | | 8.05 | 8.06 | 8.07 | 8.07 | 8.08 | 8.10 | 8.12 | 8.13 | 8.14 | 8.14 | 8.14 | 8.15 | 8.15 | 8.18 | 8.20 | 8.20 | 8.24 | 8.30 | 8.31 | 8.31 | |
| | | | 8.33 | 8.38 | 8.43 | 8.44 | 8.45 | 8.46 | 8.55 | 8.56 | 8.62 | 8.64 | 8.68 | 8.69 | 8.71 | 8.74 | 8.78 | 8.81 | 8.86 | 8.92 | 8.93 | 8.96 | |
| | | | 8.96 | 9.03 | 9.11 | 9.13 | 9.13 | 9.14 | 9.15 | 9.16 | 9.17 | 9.18 | 9.19 | 9.21 | 9.26 | 9.28 | 9.32 | 9.34 | 9.39 | 9.39 | 9.41 | 9.49 | |
| | | | 9.50 | 9.50 | 9.51 | 9.65 | 9.65 | 9.69 | 9.70 | 9.75 | 9.77 | 9.79 | 9.79 | 9.81 | 9.85 | 9.87 | 9.94 | 9.95 | 9.96 | 9.96 | 9.90 | 10.00 | 10.67 |
| 100 mg/L | W6B1 | 275 | 10.01 | 10.08 | 10.13 | 10.15 | 10.15 | 10.15 | 10.17 | 10.21 | 10.22 | 10.31 | 10.31 | 10.33 | 10.41 | 10.42 | 10.48 | 10.49 | 10.50 | 10.57 | 10.58 | 10.59 | (2.43) |
| | | | 10.65 | 10.67 | 10.69 | 10.69 | 10.77 | 10.79 | 10.81 | 10.81 | 10.81 | 10.84 | 10.87 | 10.87 | 10.93 | 10.95 | 10.96 | 11.00 | 11.07 | 11.15 | 11.18 | 11.21 | |
| | | | 11.21 | 11.22 | 11.24 | 11.25 | 11.25 | 11.27 | 11.27 | 11.29 | 11.32 | 11.33 | 11.37 | 11.45 | 11.47 | 11.49 | 11.49 | 11.53 | 11.58 | 11.58 | 11.66 | 11.66 | |
| | | | 11.88 | 11.88 | 11.90 | 11.91 | 11.99 | 12.00 | 12.00 | 12.02 | 12.03 | 12.05 | 12.06 | 12.09 | 12.18 | 12.18 | 12.23 | 12.27 | 12.28 | 12.28 | 12.33 | 12.36 | |
| | | | 12.37 | 12.37 | 12.39 | 12.39 | 12.46 | 12.56 | 12.65 | 12.67 | 12.68 | 12.72 | 12.74 | 12.74 | 12.78 | 12.80 | 12.84 | 12.85 | 12.88 | 12.89 | 12.89 | 12.96 | |
| | | | 13.00 | 13.01 | 13.06 | 13.11 | 13.13 | 13.18 | 13.20 | 13.21 | 13.22 | 13.22 | 13.26 | 13.28 | 13.28 | 13.34 | 13.36 | 13.37 | 13.40 | 13.41 | 13.43 | 13.45 | |
| | | | 13.46 | 13.50 | 13.62 | 13.62 | 13.78 | 13.79 | 13.82 | 13.84 | 13.85 | 13.87 | 13.99 | 14.11 | 14.14 | 14.16 | 14.35 | 14.36 | 14.37 | 14.55 | 14.67 | 14.67 | |
| | | | 14.74 | 14.89 | 15.01 | 15.04 | 15.25 | 15.41 | 15.48 | 15.50 | 15.92 | 16.25 | 16.34 | 16.71 | 16.86 | 17.18 | 17.78 | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | Grand Mean | | 11.26 |
| | | | | | | | | | | | | | | | | | | | | | (STD) | | (0.46) |

File Folder: 15

Item Number 1
Page 4 of 4

| | | | |
|---|---|---|---|
| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
| Electronic Lab Notebook (pages 30) | Created...... | 28-Oct-13 | KLW TS |
| Data Source: File Folder: 15 | Revised...... | 12-Feb-14 | TJS TS |
| Forms: "Zebra Mussel Lengths" | Reviewed... | 12 FEB 14 | TS |
| | Certified... | 2/19/14 | SA |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Lengths (Bottom Injection).xlsx]12h Length Data | | | |

## Zebra Mussel Lengths - Lake Carlos (Bottom Injection)

Test Article: MBI 401 SDP (*Pseudomonas fluorescens Pf*-CL 145A (SDP))
Article Lot #: 401P121163C and 401P121164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

Overall Data Summary:

| | 12 h | | |
|---|---|---|---|
| | 0 mg/L | 50 mg/L | 100 mg/L |
| Mean (mm) | 12.34 | 11.22 | 11.99 |
| (STD) | (2.70) | (2.66) | (2.88) |
| Minimum | 6.20 | 6.37 | 6.18 |
| Maximum | 20.71 | 20.06 | 18.94 |
| Grand Mean | | 11.85 | |
| (STD) | | (0.47) | |

Data Explanation:
After survival analysis, one tray from each treatment level (0, 50 and 100 mg/L) from the 12 h exposure termination time point was retained for mussel length analysis. All animals were measured for length. All lengths were reported except for those from mussels with broken shells or those < 6 mm.

Data anomalies and deviations:
NONE

File Folder: 15

Item Number 2
Page 1 of 2

Study Number: AEH-12-PSEJDO-04
Electronic Lab Notebook (pages 30)
Data Source: File Folder: 15
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP (*Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

# 12 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 mg/L | B6C1 | 184 | 6.20 | 6.49 | 6.76 | 6.96 | 6.97 | 6.99 | 7.15 | 7.88 | 7.94 | 8.02 | 8.21 | 8.27 | 8.30 | 8.34 | 8.41 | 8.54 | 8.58 | 8.58 | 8.59 | 8.65 | 12.34 (2.70) |
| | | | 8.75 | 8.79 | 8.96 | 8.98 | 9.01 | 9.02 | 9.05 | 9.07 | 9.15 | 9.19 | 9.21 | 9.55 | 9.55 | 9.59 | 9.61 | 9.64 | 9.64 | 9.66 | 9.73 | 9.78 | |
| | | | 9.85 | 9.94 | 10.06 | 10.27 | 10.33 | 10.39 | 10.42 | 10.45 | 10.53 | 10.65 | 10.66 | 10.66 | 10.72 | 10.75 | 10.83 | 10.88 | 10.93 | 10.94 | 11.11 | 11.15 | |
| | | | 11.28 | 11.32 | 11.38 | 11.39 | 11.47 | 11.50 | 11.50 | 11.51 | 11.61 | 11.63 | 11.77 | 11.78 | 11.78 | 11.82 | 11.85 | 11.87 | 11.90 | 12.02 | 12.04 | 12.04 | |
| | | | 12.06 | 12.15 | 12.16 | 12.23 | 12.23 | 12.31 | 12.37 | 12.40 | 12.41 | 12.43 | 12.55 | 12.59 | 12.62 | 12.74 | 12.79 | 12.80 | 12.81 | 12.87 | 12.88 | 12.88 | |
| | | | 12.90 | 12.93 | 12.96 | 12.97 | 13.08 | 13.08 | 13.12 | 13.14 | 13.15 | 13.16 | 13.18 | 13.23 | 13.25 | 13.28 | 13.28 | 13.31 | 13.33 | 13.35 | 13.35 | 13.43 | |
| | | | 13.53 | 13.55 | 13.55 | 13.57 | 13.61 | 13.66 | 13.67 | 13.68 | 13.73 | 13.78 | 13.78 | 13.78 | 13.83 | 13.84 | 13.85 | 13.87 | 13.90 | 13.99 | 14.05 | 14.10 | |
| | | | 14.10 | 14.15 | 14.18 | 14.24 | 14.37 | 14.39 | 14.47 | 14.53 | 14.58 | 14.58 | 14.84 | 15.12 | 15.22 | 15.24 | 15.29 | 15.33 | 15.41 | 15.42 | 15.46 | 15.49 | |
| | | | 15.56 | 15.56 | 15.59 | 15.62 | 15.70 | 15.79 | 15.86 | 15.89 | 15.98 | 16.00 | 16.06 | 16.18 | 16.29 | 16.51 | 16.74 | 16.91 | 17.15 | 17.17 | 17.21 | 17.30 | |
| | | | 17.53 | 17.74 | 17.78 | 20.71 | | | | | | | | | | | | | | | | | |
| 50 mg/L | B4B2 | 246 | 6.37 | 6.41 | 6.44 | 6.47 | 6.61 | 6.64 | 6.67 | 6.92 | 6.92 | 6.95 | 7.05 | 7.11 | 7.15 | 7.17 | 7.19 | 7.21 | 7.30 | 7.35 | 7.38 | 7.39 | 11.22 (2.66) |
| | | | 7.40 | 7.53 | 7.57 | 7.58 | 7.63 | 7.69 | 7.70 | 7.70 | 7.72 | 7.80 | 7.82 | 7.85 | 7.86 | 7.87 | 7.91 | 7.94 | 7.97 | 7.98 | 8.02 | 8.04 | |
| | | | 8.06 | 8.07 | 8.08 | 8.09 | 8.13 | 8.15 | 8.26 | 8.27 | 8.29 | 8.32 | 8.36 | 8.47 | 8.49 | 8.64 | 8.68 | 8.69 | 8.73 | 8.77 | 8.78 | 8.79 | |
| | | | 8.92 | 8.96 | 8.99 | 9.09 | 9.15 | 9.15 | 9.15 | 9.24 | 9.33 | 9.40 | 9.40 | 9.68 | 9.74 | 9.79 | 9.82 | 9.85 | 9.86 | 9.90 | 9.91 | 9.98 | |
| | | | 9.99 | 10.01 | 10.02 | 10.03 | 10.08 | 10.10 | 10.24 | 10.34 | 10.42 | 10.42 | 10.43 | 10.46 | 10.57 | 10.58 | 10.61 | 10.62 | 10.66 | 10.72 | 10.80 | 10.82 | |
| | | | 10.84 | 10.88 | 10.92 | 10.95 | 10.95 | 10.96 | 10.98 | 10.99 | 11.00 | 11.01 | 11.02 | 11.03 | 11.10 | 11.11 | 11.12 | 11.20 | 11.23 | 11.32 | 11.33 | 11.35 | |
| | | | 11.35 | 11.41 | 11.42 | 11.43 | 11.43 | 11.45 | 11.45 | 11.48 | 11.50 | 11.52 | 11.52 | 11.52 | 11.54 | 11.55 | 11.59 | 11.65 | 11.70 | 11.72 | 11.74 | 11.76 | |
| | | | 11.76 | 11.83 | 11.87 | 11.89 | 11.91 | 11.93 | 11.95 | 11.95 | 11.99 | 12.03 | 12.03 | 12.05 | 12.09 | 12.11 | 12.11 | 12.15 | 12.16 | 12.18 | 12.19 | 12.19 | |
| | | | 12.25 | 12.25 | 12.29 | 12.31 | 12.32 | 12.33 | 12.37 | 12.40 | 12.42 | 12.43 | 12.45 | 12.45 | 12.47 | 12.65 | 12.69 | 12.70 | 12.71 | 12.73 | 12.73 | 12.79 | |
| | | | 12.88 | 12.89 | 12.93 | 12.95 | 12.97 | 12.99 | 12.99 | 13.00 | 13.02 | 13.04 | 13.06 | 13.15 | 13.26 | 13.38 | 13.47 | 13.49 | 13.50 | 13.53 | 13.56 | 13.63 | |
| | | | 13.65 | 13.71 | 13.74 | 13.80 | 14.00 | 14.02 | 14.03 | 14.03 | 14.06 | 14.16 | 14.27 | 14.36 | 14.46 | 14.47 | 14.47 | 14.48 | 14.54 | 14.54 | 14.57 | 14.57 | |
| | | | 14.61 | 14.64 | 14.70 | 14.78 | 14.87 | 14.95 | 14.96 | 15.04 | 15.12 | 15.18 | 15.30 | 15.31 | 15.39 | 15.52 | 15.62 | 15.88 | 15.89 | 15.91 | 16.26 | 16.30 | |
| | | | 16.47 | 16.84 | 16.94 | 17.40 | 17.50 | 20.06 | | | | | | | | | | | | | | | |
| 100 mg/L | B9A1 | 262 | 5.18 | 6.20 | 6.24 | 6.38 | 6.46 | 6.48 | 6.56 | 6.58 | 6.78 | 7.01 | 7.05 | 7.09 | 7.11 | 7.21 | 7.23 | 7.23 | 7.28 | 7.30 | 7.44 | 7.51 | 11.99 (2.88) |
| | | | 7.60 | 7.70 | 7.81 | 7.87 | 7.88 | 7.95 | 7.96 | 8.04 | 8.06 | 8.15 | 8.15 | 8.29 | 8.30 | 8.31 | 8.38 | 8.45 | 8.50 | 8.50 | 8.55 | 8.62 | |
| | | | 8.65 | 8.67 | 8.69 | 8.70 | 8.70 | 8.74 | 8.83 | 8.83 | 8.96 | 9.03 | 9.06 | 9.07 | 9.08 | 9.15 | 9.26 | 9.29 | 9.29 | 9.29 | 9.30 | 9.41 | |
| | | | 9.46 | 9.48 | 9.61 | 9.68 | 9.68 | 9.71 | 9.74 | 9.74 | 9.81 | 9.84 | 9.95 | 10.09 | 10.09 | 10.09 | 10.13 | 10.25 | 10.27 | 10.28 | 10.47 | 10.49 | |
| | | | 10.54 | 10.54 | 10.58 | 10.61 | 10.63 | 10.69 | 10.75 | 10.79 | 10.83 | 10.84 | 10.84 | 10.84 | 10.85 | 10.86 | 10.88 | 10.95 | 10.96 | 11.04 | 11.09 | 11.10 | |
| | | | 11.13 | 11.14 | 11.18 | 11.25 | 11.28 | 11.34 | 11.39 | 11.41 | 11.42 | 11.45 | 11.47 | 11.50 | 11.52 | 11.60 | 11.62 | 11.64 | 11.67 | 11.72 | 11.75 | 11.81 | |
| | | | 11.88 | 11.90 | 11.90 | 11.92 | 11.92 | 11.93 | 12.01 | 12.04 | 12.07 | 12.13 | 12.18 | 12.21 | 12.22 | 12.22 | 12.29 | 12.33 | 12.35 | 12.40 | 12.43 | 12.45 | |
| | | | 12.48 | 12.49 | 12.50 | 12.60 | 12.60 | 12.60 | 12.60 | 12.62 | 12.63 | 12.64 | 12.67 | 12.71 | 12.73 | 12.77 | 12.78 | 12.86 | 12.88 | 12.89 | 13.03 | 13.04 | |
| | | | 13.07 | 13.08 | 13.10 | 13.15 | 13.18 | 13.18 | 13.27 | 13.31 | 13.34 | 13.35 | 13.36 | 13.39 | 13.39 | 13.42 | 13.44 | 13.46 | 13.54 | 13.54 | 13.57 | 13.63 | |
| | | | 13.63 | 13.63 | 13.70 | 13.76 | 13.77 | 13.79 | 13.80 | 13.81 | 13.93 | 14.01 | 14.03 | 14.06 | 14.12 | 14.15 | 14.20 | 14.33 | 14.34 | 14.38 | 14.45 | 14.46 | |
| | | | 14.46 | 14.50 | 14.54 | 14.55 | 14.57 | 14.59 | 14.60 | 14.60 | 14.62 | 14.67 | 14.72 | 14.72 | 14.76 | 14.81 | 14.82 | 14.88 | 14.93 | 14.95 | 14.97 | 15.01 | |
| | | | 15.02 | 15.03 | 15.04 | 15.08 | 15.09 | 15.12 | 15.17 | 15.18 | 15.21 | 15.24 | 15.25 | 15.38 | 15.38 | 15.62 | 15.65 | 15.73 | 15.81 | 15.82 | 15.91 | 15.94 | |
| | | | 15.96 | 16.03 | 16.04 | 16.05 | 16.06 | 16.13 | 16.17 | 16.21 | 16.24 | 16.55 | 16.59 | 16.67 | 16.82 | 16.84 | 16.98 | 16.99 | 17.00 | 17.06 | 17.74 | 18.18 | |
| | | | 18.66 | 18.94 | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | | 11.85 (0.47) |

Item Number 2
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File Folder: 15

| | |
|---|---|
| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (pages 30)<br>Data Source: File Folder: 16<br>Forms: "Zebra Mussel Lengths" | |
| File Name: See filenames as stated below | |

| Action | Date | Initials |
|---|---|---|
| Created...... | 28-Oct-13 | KLW |
| Revised...... | 13-Feb-14 | TJS |
| Reviewed... | 13FEB14 | TJS |
| Certified... | 2/13/14 | JAL |

## Zebra Mussel Lengths - Lake Shawano (Whole Water Column)

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

Overall Data Summary:

| | 6 h | | | 9 h | | | 12 h | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 mg/L | 50 mg/L | 100 mg/L | 0 mg/L | 50 mg/L | 100 mg/L | 0 mg/L | 50 mg/L | 100 mg/L |
| Mean (mm) | 18.33 | 18.88 | 18.80 | 18.65 | 18.67 | 18.89 | 18.67 | 17.87 | 18.28 |
| (STD) | (3.10) | (3.21) | (2.77) | (2.64) | (2.71) | (2.94) | (2.52) | (2.82) | (3.22) |
| Minimum | 7.49 | 8.13 | 11.64 | 8.33 | 11.02 | 6.23 | 13.28 | 7.57 | 6.55 |
| Maximum | 26.57 | 35.04 | 26.34 | 27.72 | 26.42 | 27.77 | 25.44 | 26.62 | 29.30 |
| Grand Mean | 18.67 | | | 18.74 | | | 18.27 | | |
| (STD) | (0.24) | | | (0.11) | | | (0.33) | | |

Data Explanation:
After survival analysis, one tray from each treatment level (0, 50 and 100 mg/L) at each exposure termination time point (6, 9 and 12 h) was retained for mussel length analysis. All animals were measured for length. All lengths were reported except for those from mussels with broken shells or those < 6 mm.

6 h Mussel Length Data
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Lengths (Whole Tank).xlsx]6h Length Data

9 h Mussel Length Data
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Lengths (Whole Tank).xlsx]9h Length Data

12 h Mussel Length Data
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Lengths (Whole Tank).xlsx]12h Length Data

Data anomalies and deviations:
NONE

File Folder: 16

Item Number 1
Page 1 of 4

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 30)
Data Source: File Folder: 16
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP (*Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

# 6 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7.49 | 7.54 | 10.86 | 11.65 | 12.62 | 14.26 | 14.45 | 15.16 | 15.34 | 15.88 | 15.94 | 16.03 | 16.21 | 16.22 | 16.28 | 16.29 | 16.46 | 16.51 | 16.61 | 16.81 | |
| 0 mg/L | W7B1 | 80 | 17.25 | 17.27 | 17.30 | 17.41 | 17.47 | 17.48 | 17.57 | 17.57 | 17.60 | 17.61 | 17.95 | 17.96 | 18.22 | 18.31 | 18.39 | 18.45 | 18.47 | 18.53 | 18.63 | 18.66 | 18.33 |
| | | | 18.72 | 18.92 | 19.02 | 19.06 | 19.16 | 19.16 | 19.19 | 19.25 | 19.26 | 19.27 | 19.32 | 19.37 | 19.47 | 19.54 | 19.58 | 19.60 | 19.67 | 19.67 | 19.88 | 19.92 | (3.10) |
| | | | 19.95 | 19.96 | 19.97 | 20.10 | 20.26 | 20.30 | 20.39 | 20.57 | 20.66 | 21.51 | 21.62 | 21.92 | 21.97 | 22.07 | 22.46 | 22.54 | 22.72 | 23.62 | 23.79 | 26.57 | |
| | | | 8.13 | 8.16 | 10.19 | 13.19 | 13.73 | 13.90 | 14.13 | 14.22 | 14.53 | 14.75 | 15.00 | 15.05 | 15.40 | 15.71 | 15.72 | 15.75 | 15.86 | 15.99 | 16.05 | 16.12 | |
| | | | 16.22 | 16.25 | 16.39 | 16.57 | 16.74 | 16.83 | 16.86 | 17.05 | 17.10 | 17.13 | 17.15 | 17.31 | 17.33 | 17.34 | 17.38 | 17.38 | 17.41 | 17.45 | 17.48 | 17.58 | |
| | | | 17.62 | 17.67 | 17.78 | 17.78 | 17.83 | 17.86 | 17.88 | 17.91 | 18.04 | 18.09 | 18.11 | 18.15 | 18.22 | 18.28 | 18.33 | 18.46 | 18.53 | 18.53 | 18.54 | 18.57 | 18.88 |
| 50 mg/L | W8C1 | 137 | 18.57 | 18.61 | 18.62 | 18.78 | 18.81 | 18.84 | 18.84 | 18.88 | 18.96 | 18.97 | 18.97 | 19.08 | 19.09 | 19.10 | 19.16 | 19.19 | 19.26 | 19.27 | 19.37 | 19.43 | (3.21) |
| | | | 19.45 | 19.46 | 19.47 | 19.55 | 19.61 | 19.62 | 19.63 | 19.72 | 19.78 | 19.83 | 19.93 | 19.95 | 19.96 | 19.97 | 19.99 | 20.00 | 20.04 | 20.11 | 20.16 | 20.19 | |
| | | | 20.30 | 20.30 | 20.33 | 20.38 | 20.38 | 20.41 | 20.48 | 20.62 | 20.64 | 20.65 | 20.65 | 20.82 | 20.83 | 20.97 | 21.00 | 21.10 | 21.16 | 21.25 | 21.39 | 21.56 | |
| | | | 21.72 | 21.82 | 21.92 | 21.97 | 22.12 | 22.28 | 22.30 | 22.58 | 22.87 | 22.90 | 22.98 | 23.70 | 24.97 | 25.17 | 27.07 | 28.74 | 35.04 | | | | |
| | | | 11.64 | 13.09 | 13.98 | 14.64 | 14.71 | 15.33 | 15.38 | 15.55 | 15.74 | 15.76 | 15.76 | 15.77 | 15.80 | 15.80 | 16.11 | 16.45 | 16.68 | 16.87 | 17.13 | 17.19 | |
| 100 mg/L | W9A1 | 73 | 17.43 | 17.46 | 17.74 | 17.86 | 17.87 | 17.92 | 17.96 | 17.98 | 18.22 | 18.25 | 18.31 | 18.33 | 18.47 | 18.47 | 18.55 | 18.59 | 18.60 | 18.67 | 18.84 | 18.96 | 18.80 |
| | | | 19.06 | 19.09 | 19.22 | 19.29 | 19.33 | 19.35 | 19.39 | 19.42 | 19.79 | 19.92 | 20.13 | 20.20 | 20.42 | 20.58 | 20.80 | 20.82 | 20.92 | 20.93 | 21.09 | 21.17 | (2.77) |
| | | | 21.29 | 21.38 | 21.42 | 21.74 | 21.87 | 21.87 | 22.05 | 22.98 | 23.27 | 23.33 | 24.01 | 26.03 | 26.34 | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | | 18.67 (0.24) |

Item Number 1
Page 2 of 4

Study Number: AEH-12-PSEJDO-04
Electronic Lab Notebook (pages 30)
Data Source: File Folder: 16
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDF [Pf-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

# 9 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 8.33 | 12.12 | 12.21 | 12.69 | 12.91 | 12.96 | 13.00 | 14.05 | 14.30 | 14.72 | 14.76 | 14.92 | 14.96 | 15.30 | 15.60 | 15.63 | 15.69 | 15.74 | 15.93 | 16.10 | |
| | | | 16.29 | 16.30 | 16.37 | 16.39 | 16.46 | 16.49 | 16.59 | 16.75 | 16.81 | 16.89 | 16.98 | 17.02 | 17.02 | 17.03 | 17.04 | 17.17 | 17.23 | 17.25 | 17.26 | 17.27 | |
| | | | 17.28 | 17.30 | 17.39 | 17.42 | 17.48 | 17.49 | 17.54 | 17.54 | 17.63 | 17.68 | 17.68 | 17.68 | 17.77 | 17.82 | 17.88 | 17.91 | 17.95 | 17.98 | 18.00 | 18.01 | |
| 0 mg/L | W1A3 | 156 | 18.03 | 18.03 | 18.05 | 18.05 | 18.07 | 18.08 | 18.09 | 18.20 | 18.20 | 18.25 | 18.43 | 18.49 | 18.51 | 18.54 | 18.66 | 18.68 | 18.72 | 18.81 | 18.85 | 18.88 | 18.65 |
| | | | 18.91 | 18.93 | 18.95 | 18.96 | 18.97 | 19.02 | 19.04 | 19.10 | 19.11 | 19.15 | 19.17 | 19.20 | 19.21 | 19.24 | 19.24 | 19.25 | 19.26 | 19.27 | 19.37 | 19.38 | (2.64) |
| | | | 19.45 | 19.46 | 19.49 | 19.55 | 19.67 | 19.69 | 19.81 | 19.81 | 19.95 | 20.09 | 20.23 | 20.27 | 20.30 | 20.32 | 20.32 | 20.33 | 20.36 | 20.43 | 20.46 | 20.50 | |
| | | | 20.62 | 20.64 | 20.64 | 20.72 | 20.73 | 20.78 | 20.82 | 20.85 | 20.89 | 20.89 | 20.90 | 20.94 | 20.96 | 21.00 | 21.03 | 21.09 | 21.15 | 21.18 | 21.20 | 21.35 | |
| | | | 21.49 | 21.51 | 21.57 | 21.66 | 21.72 | 21.90 | 21.97 | 22.00 | 22.16 | 22.24 | 22.72 | 23.04 | 23.55 | 25.71 | 26.93 | 27.72 | | | | | |
| | | | 11.02 | 12.22 | 12.62 | 13.81 | 14.12 | 14.21 | 14.25 | 14.30 | 14.73 | 14.75 | 14.92 | 14.99 | 14.99 | 15.01 | 15.03 | 15.12 | 15.12 | 15.19 | 15.20 | 15.33 | |
| | | | 15.36 | 15.69 | 15.99 | 16.01 | 16.32 | 16.33 | 16.49 | 16.51 | 16.51 | 16.53 | 16.56 | 16.58 | 16.71 | 16.75 | 16.76 | 16.78 | 16.80 | 16.82 | 16.84 | 16.97 | |
| | | | 17.03 | 17.11 | 17.18 | 17.34 | 17.35 | 17.36 | 17.41 | 17.47 | 17.50 | 17.58 | 17.58 | 17.62 | 17.63 | 17.70 | 17.73 | 17.76 | 17.77 | 17.92 | 17.92 | 17.92 | 18.67 |
| 50 mg/L | W8A1 | 147 | 17.94 | 17.97 | 17.97 | 18.03 | 18.03 | 18.10 | 18.18 | 18.45 | 18.47 | 18.56 | 18.57 | 18.59 | 18.73 | 18.78 | 18.78 | 18.92 | 18.94 | 18.96 | 18.96 | 19.02 | (2.71) |
| | | | 19.02 | 19.03 | 19.05 | 19.07 | 19.09 | 19.11 | 19.16 | 19.21 | 19.28 | 19.32 | 19.37 | 19.38 | 19.56 | 19.62 | 19.70 | 19.72 | 19.87 | 20.00 | 20.12 | 20.14 | |
| | | | 20.21 | 20.22 | 20.24 | 20.26 | 20.29 | 20.41 | 20.44 | 20.46 | 20.49 | 20.50 | 20.56 | 20.59 | 20.64 | 20.65 | 20.69 | 20.70 | 20.76 | 20.81 | 20.82 | 20.88 | |
| | | | 20.92 | 21.01 | 21.13 | 21.28 | 21.28 | 21.39 | 21.41 | 21.45 | 21.46 | 21.55 | 21.61 | 21.69 | 21.79 | 21.79 | 21.94 | 22.12 | 22.25 | 22.36 | 22.47 | 22.83 | |
| | | | 23.17 | 23.86 | 24.71 | 25.04 | 25.05 | 25.89 | 26.42 | | | | | | | | | | | | | | |
| | | | 6.23 | 7.37 | 12.34 | 14.93 | 14.94 | 15.39 | 15.39 | 15.44 | 15.45 | 15.53 | 15.77 | 15.78 | 15.82 | 15.86 | 15.98 | 16.01 | 16.03 | 16.27 | 16.28 | 16.47 | |
| | | | 16.52 | 16.63 | 16.72 | 16.78 | 16.82 | 16.84 | 16.89 | 16.89 | 16.91 | 16.92 | 16.98 | 17.00 | 17.14 | 17.20 | 17.21 | 17.30 | 17.34 | 17.41 | 17.52 | 17.53 | |
| | | | 17.68 | 17.69 | 17.73 | 17.90 | 17.90 | 17.91 | 17.93 | 17.94 | 18.13 | 18.15 | 18.26 | 18.27 | 18.40 | 18.56 | 18.63 | 18.65 | 18.67 | 18.69 | 18.71 | 18.72 | |
| 100 mg/L | W9C3 | 123 | 18.84 | 19.05 | 19.05 | 19.08 | 19.16 | 19.17 | 19.21 | 19.22 | 19.23 | 19.35 | 19.38 | 19.38 | 19.49 | 19.57 | 19.72 | 19.87 | 19.94 | 19.94 | 20.00 | 20.04 | 18.89 |
| | | | 20.16 | 20.18 | 20.19 | 20.27 | 20.44 | 20.44 | 20.45 | 20.64 | 20.76 | 20.80 | 20.80 | 20.83 | 20.90 | 20.90 | 21.00 | 21.01 | 21.04 | 21.11 | 21.18 | 21.23 | (2.94) |
| | | | 21.27 | 21.34 | 21.37 | 21.50 | 21.51 | 21.54 | 21.55 | 21.56 | 21.63 | 21.64 | 21.88 | 22.20 | 22.38 | 22.43 | 22.46 | 22.98 | 23.11 | 23.13 | 23.55 | 24.14 | |
| | | | 25.19 | 25.75 | 27.77 | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | 18.74 (0.11) |

Item Number 1
Page 3 of 4

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 30)
Data Source: File Folder: 16
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

# 12 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 13.28 | 13.74 | 14.07 | 14.22 | 14.27 | 14.28 | 14.94 | 15.04 | 15.09 | 15.18 | 15.49 | 15.63 | 15.73 | 16.03 | 16.35 | 16.37 | 16.58 | 16.77 | 16.78 | 16.81 | |
| | | | 16.87 | 17.14 | 17.15 | 17.20 | 17.20 | 17.21 | 17.25 | 17.26 | 17.26 | 17.44 | 17.45 | 17.46 | 17.48 | 17.58 | 17.65 | 17.68 | 17.69 | 17.72 | 17.73 | 17.74 | 18.67 |
| 0 mg/L | W1A2 | 99 | 17.77 | 17.86 | 18.01 | 18.02 | 18.11 | 18.15 | 18.17 | 18.24 | 18.24 | 18.33 | 18.53 | 18.57 | 18.57 | 18.67 | 18.72 | 18.72 | 18.83 | 18.85 | 18.87 | 18.91 | (2.52) |
| | | | 18.91 | 19.05 | 19.42 | 19.48 | 19.67 | 19.72 | 19.79 | 19.85 | 19.88 | 19.95 | 19.96 | 20.10 | 20.23 | 20.30 | 20.39 | 20.51 | 20.58 | 20.59 | 20.69 | 20.72 | |
| | | | 20.80 | 20.84 | 20.92 | 21.19 | 21.38 | 21.42 | 21.45 | 21.53 | 21.68 | 21.75 | 21.77 | 21.88 | 22.31 | 23.56 | 23.86 | 24.15 | 24.62 | 24.92 | 25.44 | | |
| | | | 7.57 | 8.58 | 12.73 | 13.13 | 13.29 | 13.74 | 13.82 | 14.08 | 14.43 | 14.47 | 14.78 | 14.90 | 14.98 | 14.99 | 15.38 | 15.54 | 15.61 | 15.84 | 15.88 | 15.90 | |
| | | | 15.91 | 16.01 | 16.06 | 16.08 | 16.20 | 16.36 | 16.37 | 16.56 | 16.58 | 16.77 | 16.87 | 16.94 | 16.95 | 16.99 | 17.03 | 17.04 | 17.08 | 17.15 | 17.31 | 17.37 | 17.87 |
| 50 mg/L | W2A2 | 97 | 17.46 | 17.67 | 17.75 | 17.75 | 17.79 | 17.92 | 17.92 | 17.94 | 18.07 | 18.10 | 18.16 | 18.23 | 18.34 | 18.34 | 18.36 | 18.36 | 18.40 | 18.46 | 18.52 | 18.53 | (2.82) |
| | | | 18.51 | 18.74 | 18.75 | 18.78 | 18.78 | 18.90 | 19.10 | 19.23 | 19.25 | 19.52 | 19.54 | 19.72 | 19.92 | 19.92 | 19.99 | 20.02 | 20.15 | 20.35 | 20.37 | 20.40 | |
| | | | 20.42 | 20.42 | 20.53 | 20.56 | 20.57 | 20.73 | 20.80 | 21.00 | 21.02 | 21.54 | 21.59 | 21.83 | 22.07 | 22.13 | 22.37 | 23.65 | 26.62 | | | | |
| | | | 6.55 | 6.74 | 7.99 | 9.08 | 10.53 | 11.69 | 13.15 | 13.38 | 14.07 | 14.53 | 14.63 | 14.80 | 15.08 | 15.10 | 15.19 | 15.31 | 15.39 | 15.50 | 15.67 | 15.90 | |
| | | | 15.99 | 16.11 | 16.11 | 16.35 | 16.36 | 16.49 | 16.53 | 16.74 | 16.97 | 16.97 | 16.99 | 17.17 | 17.26 | 17.26 | 17.29 | 17.34 | 17.36 | 17.47 | 17.47 | 17.56 | |
| | | | 17.61 | 17.62 | 17.65 | 17.68 | 17.72 | 17.73 | 17.79 | 17.81 | 17.84 | 18.02 | 18.05 | 18.07 | 18.15 | 18.17 | 18.25 | 18.30 | 18.32 | 18.38 | 18.41 | 18.49 | 18.28 |
| 100 mg/L | W3A3 | 129 | 18.49 | 18.52 | 18.52 | 18.52 | 18.58 | 18.60 | 18.62 | 18.63 | 18.64 | 18.75 | 18.78 | 18.81 | 18.81 | 18.83 | 18.86 | 18.98 | 19.01 | 19.01 | 19.04 | 19.08 | (3.22) |
| | | | 19.13 | 19.18 | 19.21 | 19.22 | 19.29 | 19.39 | 19.40 | 19.44 | 19.47 | 19.50 | 19.51 | 19.52 | 19.56 | 19.56 | 19.58 | 19.63 | 19.66 | 19.70 | 19.79 | 19.81 | |
| | | | 19.84 | 20.06 | 20.18 | 20.22 | 20.33 | 20.42 | 20.47 | 20.51 | 20.62 | 20.66 | 20.83 | 20.83 | 20.91 | 21.01 | 21.35 | 21.46 | 21.66 | 21.82 | 21.88 | 22.17 | |
| | | | 22.73 | 22.74 | 22.76 | 22.80 | 22.82 | 23.57 | 23.66 | 27.97 | 29.30 | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | 18.27 (0.33) |

Item Number 1
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File Folder: 16

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 30) | Created...... | 28-Oct-13 | KLW TJS |
| Data Source: File Folder: 16 | Revised...... | 12-Feb-14 | TJS TJS |
| Forms: "Zebra Mussel Lengths" | Reviewed... | 12FEB14 | TJS |
| | Certified... | 2/19/14 | JR |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Lengths (Bottom Injection).xlsx]12h Length Data

### Zebra Mussel Lengths - Lake Shawano (Bottom Injection)

Test Article: MBI 401 SDP (*Pseudomonas fluorescens Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

Overall Data Summary:

| | 12 h | | |
|---|---|---|---|
| | 0 mg/L | 50 mg/L | 100 mg/L |
| Mean (mm) | 18.92 | 18.59 | 18.52 |
| (STD) | (2.58) | (2.71) | (2.81) |
| Minimum | 13.68 | 9.86 | 9.00 |
| Maximum | 30.12 | 29.01 | 30.72 |
| Grand Mean | | 18.68 | |
| (STD) | | (0.18) | |

Data Explanation:
After survival analysis, one tray from each treatment level (0, 50 and 100 mg/L) from the 12 h exposure termination time point was retained for mussel length analysis. All animals were measured for length. All lengths were reported except for those from mussels with broken shells or those < 6 mm.

Data anomalies and deviations:
NONE

File Folder: 16

Item Number 2
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 90)
Data Source: File Folder: 16
Forms: "Zebra Mussel Lengths"

Test Article: MBI 401 SDP [Pf-CL 145A (SDP)]
Article Lot #: 401P121 63C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

# 12 hour Mussel Length Data

| Treatment Level | Sample ID | N | Lengths (mm) | | | | | | | | | | | | | | | | | | | | Mean (STD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 mg/L | B1C3 | 145 | 13.68 | 13.72 | 13.86 | 14.17 | 14.20 | 14.78 | 14.91 | 15.00 | 15.27 | 15.64 | 15.88 | 15.06 | 16.10 | 16.11 | 16.18 | 16.19 | 16.24 | 16.25 | 16.37 | 16.38 | 18.92 (2.53) |
| | | | 16.46 | 16.53 | 16.55 | 16.56 | 16.71 | 16.74 | 16.91 | 16.98 | 17.05 | 17.08 | 17.14 | 17.16 | 17.20 | 17.20 | 17.21 | 17.23 | 17.31 | 17.45 | 17.47 | 17.58 | |
| | | | 17.59 | 17.65 | 17.59 | 17.70 | 17.78 | 17.78 | 17.82 | 17.92 | 17.92 | 17.92 | 17.94 | 17.96 | 17.96 | 17.96 | 17.98 | 18.00 | 18.04 | 18.08 | 18.09 | 18.13 | |
| | | | 18.16 | 18.28 | 18.37 | 18.40 | 18.41 | 18.45 | 18.43 | 18.61 | 18.61 | 18.66 | 18.72 | 18.73 | 18.74 | 18.74 | 18.84 | 18.86 | 18.90 | 18.93 | 18.97 | 18.98 | |
| | | | 19.03 | 19.08 | 19.15 | 19.26 | 19.30 | 19.31 | 19.32 | 19.34 | 19.36 | 19.37 | 19.48 | 19.49 | 19.49 | 19.56 | 19.61 | 19.66 | 19.71 | 19.71 | 19.71 | 19.74 | |
| | | | 19.75 | 19.76 | 19.76 | 19.80 | 19.80 | 19.81 | 19.92 | 20.03 | 20.12 | 20.12 | 20.16 | 20.27 | 20.31 | 20.41 | 20.43 | 20.44 | 20.44 | 20.54 | 20.58 | 20.61 | |
| | | | 20.64 | 20.66 | 20.70 | 20.74 | 20.80 | 21.04 | 21.13 | 21.13 | 21.54 | 21.75 | 21.88 | 22.05 | 22.08 | 22.30 | 22.39 | 22.79 | 22.92 | 23.14 | 24.55 | 25.25 | |
| | | | 25.46 | 26.12 | 26.42 | 26.55 | 30.12 | | | | | | | | | | | | | | | | |
| 50 mg/L | B3C2 | 111 | 9.86 | 10.42 | 11.40 | 13.28 | 13.77 | 14.04 | 14.24 | 14.45 | 14.43 | 15.17 | 15.24 | 15.34 | 15.49 | 15.74 | 15.93 | 15.98 | 16.00 | 16.07 | 16.35 | 16.42 | 18.59 (2.71) |
| | | | 16.57 | 16.60 | 16.61 | 16.68 | 16.89 | 17.06 | 17.23 | 17.25 | 17.26 | 17.37 | 17.40 | 17.43 | 17.49 | 17.58 | 17.64 | 17.71 | 17.89 | 17.89 | 17.98 | 17.98 | |
| | | | 18.04 | 18.12 | 18.18 | 18.20 | 18.21 | 18.22 | 18.30 | 18.31 | 18.34 | 18.38 | 16.75 | 18.76 | 18.77 | 18.82 | 18.86 | 18.87 | 18.94 | 18.96 | 18.99 | 19.01 | |
| | | | 19.02 | 19.06 | 19.15 | 19.15 | 19.17 | 19.20 | 19.23 | 19.25 | 19.32 | 19.32 | 19.33 | 19.42 | 19.47 | 19.49 | 19.54 | 19.58 | 19.70 | 19.71 | 19.81 | 19.90 | |
| | | | 20.03 | 20.17 | 20.25 | 20.30 | 20.38 | 20.43 | 20.47 | 20.49 | 20.51 | 20.58 | 20.62 | 20.99 | 21.03 | 21.07 | 21.15 | 21.17 | 21.19 | 21.26 | 21.41 | 21.52 | |
| | | | 21.61 | 21.75 | 21.90 | 22.17 | 22.29 | 22.34 | 22.51 | 22.75 | 22.97 | 23.80 | 29.01 | | | | | | | | | | |
| 100 mg/L | B4B3 | 129 | 9.00 | 12.01 | 13.46 | 13.77 | 13.78 | 13.98 | 14.19 | 14.21 | 14.51 | 14.61 | 14.75 | 15.08 | 15.19 | 15.23 | 15.58 | 15.76 | 15.79 | 15.86 | 15.88 | 15.97 | 18.52 (2.81) |
| | | | 16.07 | 16.11 | 16.22 | 16.47 | 16.50 | 16.57 | 16.74 | 16.83 | 16.97 | 16.99 | 17.03 | 17.08 | 17.09 | 17.10 | 17.14 | 17.17 | 17.29 | 17.30 | 17.31 | 17.32 | |
| | | | 17.34 | 17.37 | 17.39 | 17.40 | 17.44 | 17.45 | 17.54 | 17.56 | 17.57 | 17.61 | 17.76 | 17.90 | 17.95 | 17.98 | 17.99 | 18.00 | 18.01 | 18.11 | 18.13 | 18.16 | |
| | | | 18.31 | 18.37 | 18.40 | 18.41 | 18.43 | 18.47 | 18.49 | 18.51 | 18.56 | 18.67 | 18.73 | 18.75 | 18.75 | 18.76 | 18.77 | 18.84 | 18.89 | 18.91 | 18.97 | 19.01 | |
| | | | 19.06 | 19.07 | 19.10 | 19.13 | 19.24 | 19.30 | 19.31 | 19.37 | 19.42 | 19.44 | 19.45 | 19.46 | 19.62 | 19.67 | 19.84 | 20.00 | 20.03 | 20.14 | 20.34 | 20.36 | |
| | | | 20.44 | 20.48 | 20.62 | 20.63 | 20.81 | 20.82 | 20.82 | 20.86 | 20.98 | 21.13 | 21.22 | 21.28 | 21.50 | 21.53 | 21.72 | 21.75 | 21.79 | 21.87 | 21.88 | 21.92 | |
| | | | 22.42 | 22.48 | 22.61 | 22.62 | 23.01 | 23.36 | 23.47 | 28.73 | 30.72 | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | Grand Mean (STD) | 18.68 (0.15) |

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File Folder: 16

# Appendix 6. Water Quality

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (page 10) | Created...... | 19-Oct-13 | KLW KW |
| Data Source: File Folder: 9b | Revised...... | 10-Dec-14 | KLW KW |
| Forms: See form names as stated below | Reviewed... | 10DEC14 | KW |
| | Certified... | 12/10/14 | JI |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Carlos Water Chem (Whole Tank) 11-26-14.xlsx]Pre-Exposure Water Chem | | | |

**Pre-Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

Data Explanation:
Forms titled "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation": Conductivity, hardness, and alkalinity were measured prior to dosing. Samples were collected from both headboxes used to fill individual tanks. Samples were analyzed in triplicate.

Forms titled "Water Quality - Temperature (°C) Measurements", "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements": Temperature, pH and dissolved oxygen levels were measured prior to dosing. Measurements were observed in each exposure tank.

Data anomalies and deviations:
NONE

File Folder: 9b

Item Number 1
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 10)
Data Source: File Folder: 9b
Forms: See table footnotes

Test Article: MBI 401 SDP [Pf-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

## Pre-Exposure Water Chemistry

| Headbox ID | Replicate | Conductivity (µS) | Hardness (mg/L of $CaCO_3$) | Alkalinity (mg/L of $CaCO_3$) |
|---|---|---|---|---|
| 1 | 1 | 394 | 178 | 163 |
| | 2 | 396 | 176 | 163 |
| | 3 | 394 | 176 | 162 |
| 2 | 1 | 396 | 178 | 163 |
| | 2 | 393 | 178 | 162 |
| | 3 | 397 | 176 | 162 |
| Mean | | 395 | 177 | 163 |
| STD | | 1 | 1 | 1 |
| Minimum | | 393 | 176 | 162 |
| Maximum | | 397 | 178 | 163 |

Data Forms: "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation"

| Treatment Level | Test Tank ID | DO (mg/L) | pH[1] | Temp. (°C) |
|---|---|---|---|---|
| 0 | 2 | 8.74 | 8.64 | 22.1 |
| | 3 | 8.76 | 8.63 | 22.2 |
| | 5 | 8.80 | 8.62 | 22.2 |
| Mean | | 8.77 | 8.63 | 22.17 |
| Std | | 0.02 | 0.01 | 0.05 |
| 50 | 1 | 8.76 | 8.65 | 22.1 |
| | 4 | 8.75 | 8.63 | 22.2 |
| | 8 | 8.77 | 8.61 | 22.1 |
| Mean | | 8.76 | 8.63 | 22.13 |
| Std | | 0.01 | 0.02 | 0.05 |
| 100 | 6 | 8.75 | 8.60 | 22.2 |
| | 7 | 8.76 | 8.61 | 22.2 |
| | 9 | 8.78 | 8.63 | 22.2 |
| Mean | | 8.76 | 8.61 | 22.20 |
| Std | | 0.01 | 0.01 | 0.00 |
| Grand Mean | | 8.76 | 8.62 | 22.17 |
| STD | | 0.00 | 3.11 | 0.03 |
| Minimum | | 8.74 | 8.60 | 22.10 |
| Maximum | | 8.80 | 8.65 | 22.20 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

Data Forms: "Water Quality - Temperature (°C) Measurements, "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements"

Item Number 1
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File Folder: 9b

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (page 11) | Created...... | 19-Oct-13 | KLW KW |
| Data Source: File Folder: 9b | Revised...... | 10-Dec-14 | KLW KW |
| Forms: "Water Quality - Temperature (°C) Measurements" | Reviewed... | 10 Dec 14 | KW |
| "Water Quality - pH Measurements" | Certified... | 12/10/14 | Sn |
| "Water Quality - Dissolved Oxygen (mg/L) Measurements" | | | |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Carlos Water Chem (Whole Tank) 11-26-14.xlsx]Exposure Water Chem | | | |

**Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

Data Explanation:
Water chemistry measurements (dissolved oxygen, pH and temperature) were observed for all test tanks at 0, 3, 6, 9 and 12 h after dosing.
NOTE: 0 h measurements were observed from 30 minutes to 1 h after dosing.

Data anomalies and deviations:
NONE

File Folder: 9b

Item Number 2
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 11)
Data Source: File Folder: 9b
Forms: "Water Quality - Temperature (°C) Measurements"
"Water Quality - pH Measurements"
"Water Quality - Dissolved Oxygen (mg/L) Measurements"

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

## Exposure Water Chemistry

| Treatment Level (mg/L) | Test Tank ID | 0 hour DO (mg/L) | 0 hour pH[1] | 0 hour Temp. (°C) | 3 Hour DO (mg/L) | 3 Hour pH[1] | 3 Hour Temp. (°C) | 6 Hour DO (mg/L) | 6 Hour pH[1] | 6 Hour Temp. (°C) | 9 Hour DO (mg/L) | 9 Hour pH[1] | 9 Hour Temp. (°C) | 12 Hour DO (mg/L) | 12 Hour pH[1] | 12 Hour Temp. (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2 | 8.68 | 8.60 | 22.3 | 8.47 | 8.56 | 22.4 | 8.46 | 8.55 | 22.5 | 8.24 | 8.25 | 22.5 | 8.12 | 8.54 | 22.5 |
| | 3 | 8.72 | 8.62 | 22.3 | 8.57 | 8.57 | 22.4 | 8.50 | 8.54 | 22.5 | 8.22 | 8.29 | 22.5 | 8.11 | 8.56 | 22.5 |
| | 5 | 8.77 | 8.63 | 22.4 | 8.70 | 8.58 | 22.5 | 8.56 | 8.58 | 22.6 | 8.30 | 8.31 | 22.4 | 8.10 | 8.57 | 22.4 |
| Mean | | 8.72 | 8.62 | 22.3 | 8.58 | 8.57 | 22.4 | 8.51 | 8.56 | 22.5 | 8.25 | 8.28 | 22.5 | 8.11 | 8.56 | 22.5 |
| STD | | 0.04 | 0.01 | 0.05 | 0.09 | 0.01 | 0.05 | 0.04 | 0.02 | 0.05 | 0.03 | 0.02 | 0.05 | 0.01 | 0.01 | 0.05 |
| 50 | 1 | 8.68 | 8.57 | 22.2 | 8.40 | 8.49 | 22.4 | 8.17 | 8.47 | 22.5 | 7.86 | 8.20 | 22.5 | 6.75 | 8.33 | 22.5 |
| | 4 | 8.65 | 8.57 | 22.3 | 8.43 | 8.48 | 22.5 | 8.26 | 8.49 | 22.5 | 7.86 | 8.20 | 22.6 | 6.68 | 8.34 | 22.5 |
| | 8 | 8.64 | 8.57 | 22.3 | 8.42 | 8.47 | 22.4 | 8.19 | 8.48 | 22.5 | 7.67 | 8.21 | 22.4 | 6.44 | 8.35 | 22.4 |
| Mean | | 8.66 | 8.57 | 22.3 | 8.42 | 8.48 | 22.4 | 8.21 | 8.48 | 22.5 | 7.80 | 8.20 | 22.5 | 6.62 | 8.34 | 22.5 |
| STD | | 0.02 | 0.00 | 0.05 | 0.01 | 0.01 | 0.05 | 0.04 | 0.01 | 0.00 | 0.09 | 0.00 | 0.08 | 0.13 | 0.01 | 0.05 |
| 100 | 6 | 8.64 | 8.53 | 22.3 | 8.43 | 8.39 | 22.5 | 8.20 | 8.37 | 22.5 | 7.84 | 8.12 | 22.4 | 6.93 | 8.21 | 22.4 |
| | 7 | 8.63 | 8.53 | 22.3 | 8.39 | 8.38 | 22.4 | 7.97 | 8.39 | 22.4 | 7.70 | 8.12 | 22.3 | 6.84 | 8.24 | 22.3 |
| | 9 | 8.65 | 8.53 | 22.3 | 8.33 | 8.37 | 22.4 | 8.02 | 8.39 | 22.5 | 7.68 | 8.12 | 22.5 | 6.76 | 8.23 | 22.5 |
| Mean | | 8.64 | 8.53 | 22.3 | 8.38 | 8.38 | 22.4 | 8.06 | 8.38 | 22.5 | 7.74 | 8.12 | 22.4 | 6.84 | 8.23 | 22.4 |
| STD | | 0.01 | 0.00 | 0.00 | 0.04 | 0.01 | 0.05 | 0.10 | 0.01 | 0.05 | 0.07 | 0.00 | 0.08 | 0.07 | 0.01 | 0.08 |
| Grand Mean | | 8.67 | 8.57 | 22.3 | 8.46 | 8.48 | 22.4 | 8.26 | 8.48 | 22.5 | 7.93 | 8.21 | 22.5 | 7.19 | 8.40 | 22.4 |
| STD | | 0.04 | 0.04 | 0.03 | 0.09 | 0.08 | 0.00 | 0.18 | 0.07 | 0.03 | 0.23 | 0.07 | 0.04 | 0.66 | 0.14 | 0.03 |
| Minimum | | 8.63 | 8.53 | 22.2 | 8.33 | 8.37 | 22.4 | 7.97 | 8.37 | 22.4 | 7.67 | 8.12 | 22.3 | 6.44 | 8.21 | 22.3 |
| Maximum | | 8.77 | 8.63 | 22.4 | 8.70 | 8.58 | 22.5 | 8.56 | 8.58 | 22.6 | 8.30 | 8.31 | 22.6 | 8.12 | 8.57 | 22.5 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

File Folder: 9b

Item Number 2
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| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (page 11)<br>TAN Data Source: LTRMP Report (File Folder 17)<br>pH and Temperature Data Source: Water Quality Forms (File Folder 9b) | Action | Date | Initials |
|---|---|---|---|
| | Created...... | 19-Oct-13 | KLW KW |
| | Revised...... | 10-Dec-14 | KLW KW |
| | Reviewed... | 10DEC14 | KW |
| | Certified...... | 12/10/14 | JA |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Carlos Water Chem (Whole Tank) 11-26-14.xlsx]Ammonia Data

**Exposure Un-ionized Ammonia**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

Data Explanation:
1) Water samples were collected at 6, 9 and 12 h from each exposure tank. Samples were 0.45 µm filtered, acidified with sulfuric acid, and analyzed by the 4500-$NH_3$ G. Automated Phenate Method (Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005) on a Technicon Autoanalyzer II by the UMESC water quality laboratory.
2) The un-ionized ammonia fractions were calculated using the sample pH and temperature according to the formula in Emerson et al. (1975).

Data Anomalies and Deviations:
NONE

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File Folder: 9b

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 11)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 9b)

Test Article: MBI 401 SDP (*Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

## Exposure Un-Ionized Ammonia

| Sample Time | Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|---|
| 6 | 0 | 2 | 8.55 | 22.5 | 0.174 | 0.144 | 0.025 |
| | | 3 | 8.54 | 22.5 | 0.172 | 0.141 | 0.024 |
| | | 5 | 8.58 | 22.6 | 0.167 | 0.154 | 0.026 |
| | Mean | | 8.56 | 22.5 | 0.171 | 0.146 | 0.025 |
| | std | | 0.02 | 0.0 | 0.003 | 0.005 | 0.001 |
| | 50 | 1 | 8.47 | 22.5 | 0.225 | 0.123 | 0.028 |
| | | 4 | 8.49 | 22.5 | 0.229 | 0.128 | 0.029 |
| | | 8 | 8.48 | 22.5 | 0.215 | 0.125 | 0.027 |
| | Mean | | 8.48 | 22.5 | 0.223 | 0.125 | 0.028 |
| | std | | 0.01 | 0.0 | 0.006 | 0.002 | 0.001 |
| | 100 | 6 | 8.37 | 22.5 | 0.262 | 0.100 | 0.026 |
| | | 7 | 8.39 | 22.4 | 0.272 | 0.104 | 0.028 |
| | | 9 | 8.39 | 22.5 | 0.270 | 0.104 | 0.028 |
| | Mean | | 8.38 | 22.5 | 0.268 | 0.103 | 0.028 |
| | std | | 0.01 | 0.0 | 0.004 | 0.002 | 0.001 |
| 9 | 0 | 2 | 8.25 | 22.5 | 0.171 | 0.078 | 0.013 |
| | | 3 | 8.29 | 22.5 | 0.169 | 0.085 | 0.014 |
| | | 5 | 8.31 | 22.4 | 0.180 | 0.088 | 0.015 |
| | Mean | | 8.28 | 22.5 | 0.173 | 0.083 | 0.014 |
| | std | | 0.02 | 0.0 | 0.005 | 0.004 | 0.001 |
| | 50 | 1 | 8.20 | 22.5 | 0.225 | 0.070 | 0.016 |
| | | 4 | 8.20 | 22.6 | 0.223 | 0.070 | 0.016 |
| | | 8 | 8.21 | 22.4 | 0.231 | 0.071 | 0.016 |
| | Mean | | 8.20 | 22.5 | 0.226 | 0.070 | 0.016 |
| | std | | 0.00 | 0.1 | 0.003 | 0.000 | 0.000 |
| | 100 | 6 | 8.12 | 22.4 | 0.273 | 0.058 | 0.016 |
| | | 7 | 8.12 | 22.3 | 0.273 | 0.058 | 0.015 |
| | | 9 | 8.12 | 22.5 | 0.273 | 0.059 | 0.016 |
| | Mean | | 8.12 | 22.4 | 0.273 | 0.058 | 0.016 |
| | std | | 0.00 | 0.1 | 0.000 | 0.000 | 0.000 |
| 12 | 0 | 2 | 8.54 | 22.5 | 0.224 | 0.141 | 0.032 |
| | | 3 | 8.56 | 22.5 | 0.254 | 0.147 | 0.037 |
| | | 5 | 8.57 | 22.4 | 0.256 | 0.149 | 0.038 |
| | Mean | | 8.56 | 22.5 | 0.245 | 0.146 | 0.036 |
| | std | | 0.01 | 0.0 | 0.015 | 0.003 | 0.003 |
| | 50 | 1 | 8.33 | 22.5 | 0.318 | 0.092 | 0.029 |
| | | 4 | 8.34 | 22.5 | 0.331 | 0.094 | 0.031 |
| | | 8 | 8.35 | 22.4 | 0.334 | 0.095 | 0.032 |
| | Mean | | 8.34 | 22.5 | 0.328 | 0.094 | 0.031 |
| | std | | 0.01 | 0.0 | 0.007 | 0.001 | 0.001 |
| | 100 | 6 | 8.21 | 22.4 | 0.354 | 0.071 | 0.025 |
| | | 7 | 8.24 | 22.3 | 0.339 | 0.075 | 0.025 |
| | | 9 | 8.23 | 22.5 | 0.333 | 0.075 | 0.025 |
| | Mean | | 8.23 | 22.4 | 0.342 | 0.074 | 0.025 |
| | std | | 0.01 | 0.1 | 0.009 | 0.002 | 0.000 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction ($f$) is calculated based on the following formula (Emerson et al. 1975) $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{272992}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15 + T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018+\left(\frac{272992}{(273.15+T_c)}\right)\right)-pH}\right]+1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia = $f$ * TAN (mg/L)

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | B | C | D | E | F | G | H |

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 11)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 9b)

Test Article: MBI 401 SDP (Pf-CL 145A (SDP))
Article Lot #: 401P121690 and 401P12164C Mb
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

## Exposure Un-Ionized Ammonia

| Sample Time | Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-Ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|---|
| | | 2 | 8.55 | 22.5 | 0.174 | x=1/(10^(0.09018+(2729.92/(273.15+E12))-D12)+1) | x=G12*F12 |
| | 0 | 3 | 8.54 | 22.5 | 0.172 | x=1/(10^(0.09018+(2729.92/(273.15+E13))-D13)+1) | x=G13*F13 |
| | | 5 | 8.58 | 22.6 | 0.167 | x=1/(10^(0.09018+(2729.92/(273.15+E14))-D14)+1) | x=G14*F14 |
| | Mean | | x=(LOG10((10^D12+10^D13+10^D14)/3)) | x=AVERAGE(E12:E14) | x=AVERAGE(F12:F14) | x=AVERAGE(G12:G14) | x=AVERAGE(H12:H14) |
| | std | | x=STDEV.P(D12:D14) | x=STDEV.P(E12:E14) | x=STDEV.P(F12:F14) | x=STDEV.P(G12:G14) | x=STDEV.P(H12:H14) |
| | | 1 | 8.47 | 22.5 | 0.225 | x=1/(10^(0.09018+(2729.92/(273.15+E17))-D17)+1) | x=G17*F17 |
| 6 | 50 | 4 | 8.49 | 22.5 | 0.229 | x=1/(10^(0.09018+(2729.92/(273.15+E18))-D18)+1) | x=G18*F18 |
| | | 8 | 8.48 | 22.5 | 0.215 | x=1/(10^(0.09018+(2729.92/(273.15+E19))-D19)+1) | x=G19*F19 |
| | Mean | | x=(LOG10((10^D17+10^D18+10^D19)/3)) | x=AVERAGE(E17:E19) | x=AVERAGE(F17:F19) | x=AVERAGE(G17:G19) | x=AVERAGE(H17:H19) |
| | std | | x=STDEV.P(D17:D19) | x=STDEV.P(E17:E19) | x=STDEV.P(F17:F19) | x=STDEV.P(G17:G19) | x=STDEV.P(H17:H19) |
| | | 6 | 8.37 | 22.5 | 0.262 | x=1/(10^(0.09018+(2729.92/(273.15+E22))-D22)+1) | x=G22*F22 |
| | 100 | 7 | 8.39 | 22.4 | 0.272 | x=1/(10^(0.09018+(2729.92/(273.15+E23))-D23)+1) | x=G23*F23 |
| | | 9 | 8.39 | 22.5 | 0.270 | x=1/(10^(0.09018+(2729.92/(273.15+E24))-D24)+1) | x=G24*F24 |
| | Mean | | x=(LOG10((10^D22+10^D23+10^D24)/3)) | x=AVERAGE(E22:E24) | x=AVERAGE(F22:F24) | x=AVERAGE(G22:G24) | x=AVERAGE(H22:H24) |
| | std | | x=STDEV.P(D22:D24) | x=STDEV.P(E22:E24) | x=STDEV.P(F22:F24) | x=STDEV.P(G22:G24) | x=STDEV.P(H22:H24) |
| | | 2 | 8.25 | 22.5 | 0.171 | x=1/(10^(0.09018+(2729.92/(273.15+E27))-D27)+1) | x=G27*F27 |
| | 0 | 3 | 8.29 | 22.5 | 0.169 | x=1/(10^(0.09018+(2729.92/(273.15+E28))-D28)+1) | x=G28*F28 |
| | | 5 | 8.31 | 22.4 | 0.180 | x=1/(10^(0.09018+(2729.92/(273.15+E29))-D29)+1) | x=G29*F29 |
| | Mean | | x=(LOG10((10^D27+10^D28+10^D29)/3)) | x=AVERAGE(E27:E29) | x=AVERAGE(F27:F29) | x=AVERAGE(G27:G29) | x=AVERAGE(H27:H29) |
| | std | | x=STDEV.P(D27:D29) | x=STDEV.P(E27:E29) | x=STDEV.P(F27:F29) | x=STDEV.P(G27:G29) | x=STDEV.P(H27:H29) |
| | | 1 | 8.20 | 22.5 | 0.225 | x=1/(10^(0.09018+(2729.92/(273.15+E32))-D32)+1) | x=G32*F32 |
| 9 | 50 | 4 | 8.20 | 22.6 | 0.223 | x=1/(10^(0.09018+(2729.92/(273.15+E33))-D33)+1) | x=G33*F33 |
| | | 8 | 8.21 | 22.4 | 0.231 | x=1/(10^(0.09018+(2729.92/(273.15+E34))-D34)+1) | x=G34*F34 |
| | Mean | | x=(LOG10((10^D32+10^D33+10^D34)/3)) | x=AVERAGE(E32:E34) | x=AVERAGE(F32:F34) | x=AVERAGE(G32:G34) | x=AVERAGE(H32:H34) |
| | std | | x=STDEV.P(D32:D34) | x=STDEV.P(E32:E34) | x=STDEV.P(F32:F34) | x=STDEV.P(G32:G34) | x=STDEV.P(H32:H34) |
| | | 6 | 8.12 | 22.4 | 0.273 | x=1/(10^(0.09018+(2729.92/(273.15+E37))-D37)+1) | x=G37*F37 |
| | 100 | 7 | 8.12 | 22.3 | 0.273 | x=1/(10^(0.09018+(2729.92/(273.15+E38))-D38)+1) | x=G38*F38 |
| | | 9 | 8.12 | 22.5 | 0.273 | x=1/(10^(0.09018+(2729.92/(273.15+E39))-D39)+1) | x=G39*F39 |
| | Mean | | x=(LOG10((10^D37+10^D38+10^D39)/3)) | x=AVERAGE(E37:E39) | x=AVERAGE(F37:F39) | x=AVERAGE(G37:G39) | x=AVERAGE(H37:H39) |
| | std | | x=STDEV.P(D37:D39) | x=STDEV.P(E37:E39) | x=STDEV.P(F37:F39) | x=STDEV.P(G37:G39) | x=STDEV.P(H37:H39) |
| | | 2 | 8.54 | 22.5 | 0.224 | x=1/(10^(0.09018+(2729.92/(273.15+E42))-D42)+1) | x=G42*F42 |
| | 0 | 3 | 8.56 | 22.5 | 0.254 | x=1/(10^(0.09018+(2729.92/(273.15+E43))-D43)+1) | x=G43*F43 |
| | | 5 | 8.57 | 22.4 | 0.256 | x=1/(10^(0.09018+(2729.92/(273.15+E44))-D44)+1) | x=G44*F44 |
| | Mean | | x=(LOG10((10^D42+10^D43+10^D44)/3)) | x=AVERAGE(E42:E44) | x=AVERAGE(F42:F44) | x=AVERAGE(G42:G44) | x=AVERAGE(H42:H44) |
| | std | | x=STDEV.P(D42:D44) | x=STDEV.P(E42:E44) | x=STDEV.P(F42:F44) | x=STDEV.P(G42:G44) | x=STDEV.P(H42:H44) |
| | | 1 | 8.33 | 22.5 | 0.318 | x=1/(10^(0.09018+(2729.92/(273.15+E47))-D47)+1) | x=G47*F47 |
| 12 | 50 | 4 | 8.34 | 22.5 | 0.331 | x=1/(10^(0.09018+(2729.92/(273.15+E48))-D48)+1) | x=G48*F48 |
| | | 8 | 8.35 | 22.4 | 0.334 | x=1/(10^(0.09018+(2729.92/(273.15+E49))-D49)+1) | x=G49*F49 |
| | Mean | | x=(LOG10((10^D47+10^D48+10^D49)/3)) | x=AVERAGE(E47:E49) | x=AVERAGE(F47:F49) | x=AVERAGE(G47:G49) | x=AVERAGE(H47:H49) |
| | std | | x=STDEV.P(D47:D49) | x=STDEV.P(E47:E49) | x=STDEV.P(F47:F49) | x=STDEV.P(G47:G49) | x=STDEV.P(H47:H49) |
| | | 6 | 8.21 | 22.4 | 0.354 | x=1/(10^(0.09018+(2729.92/(273.15+E52))-D52)+1) | x=G52*F52 |
| | 100 | 7 | 8.24 | 22.3 | 0.339 | x=1/(10^(0.09018+(2729.92/(273.15+E53))-D53)+1) | x=G53*F53 |
| | | 9 | 8.23 | 22.5 | 0.333 | x=1/(10^(0.09018+(2729.92/(273.15+E54))-D54)+1) | x=G54*F54 |
| | Mean | | x=(LOG10((10^D52+10^D53+10^D54)/3)) | x=AVERAGE(E52:E54) | x=AVERAGE(F52:F54) | x=AVERAGE(G52:G54) | x=AVERAGE(H52:H54) |
| | std | | x=STDEV.P(D52:D54) | x=STDEV.P(E52:E54) | x=STDEV.P(F52:F54) | x=STDEV.P(G52:G54) | x=STDEV.P(H52:H54) |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction ($f$) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa - pH}) + 1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15 + T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018 + \left(\frac{2729.92}{273.15 + T_c}\right)\right) - pH}\right] + 1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia x= f * TAN (mg/L)

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File Folder: 9b

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (page 21) | Created...... | 19-Oct-13 | KLW |
| Data Source: File Folder: 11b | Revised...... | 10-Dec-14 | KLW |
| Forms: See form names as stated below | Reviewed... | 10Dec14 | KW |
| | Certified... | 12/10/14 | JW |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Shawano Water Chem (Whole Tank) 11-26-14.xlsx]Pre-Exposure Water Chem

**Pre-Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

Data Explanation:
Forms titled "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation": Conductivity, hardness, and alkalinity were measured prior to dosing. Samples were collected from both headboxes used to fill individual tanks. Samples were analyzed in triplicate.

Forms titled "Water Quality - Temperature (°C) Measurements", "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements": Temperature, pH and dissolved oxygen levels were measured prior to dosing. Measurements were observed in each exposure tank.

Data anomalies and deviations:
NONE

File Folder: 11b

Item Number 1
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## Pre-Exposure Water Chemistry

| Headbox ID | Replicate | Conductivity (µS) | Hardness (mg/L of $CaCO_3$) | Alkalinity (mg/L of $CaCO_3$) |
|---|---|---|---|---|
| 1 | 1 | 248 | 118 | 105 |
| | 2 | 248 | 118 | 106 |
| | 3 | 248 | 118 | 105 |
| 2 | 1 | 245 | 118 | 106 |
| | 2 | 247 | 120 | 105 |
| | 3 | 251 | 118 | 105 |
| Mean | | 248 | 118 | 105 |
| (STD) | | (2) | (1) | (0) |
| Minimum | | 245 | 118 | 105 |
| Maximum | | 251 | 120 | 106 |

Data Forms: "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation"

| Treatment Level | Test Tank ID | DO (mg/L) | pH[1] | Temp. (°C) |
|---|---|---|---|---|
| 0 | 1 | 7.32 | 9.31 | 22.6 |
| | 4 | 7.36 | 9.33 | 22.6 |
| | 7 | 7.35 | 9.34 | 22.6 |
| Mean | | 7.34 | 9.33 | 22.60 |
| std | | 0.02 | 0.01 | 0.00 |
| 50 | 2 | 7.37 | 9.33 | 22.7 |
| | 5 | 7.38 | 9.33 | 22.6 |
| | 8 | 7.34 | 9.34 | 22.6 |
| Mean | | 7.36 | 9.33 | 22.63 |
| std | | 0.02 | 0.00 | 0.05 |
| 100 | 3 | 7.35 | 9.34 | 22.6 |
| | 6 | 7.36 | 9.34 | 22.6 |
| | 9 | 7.37 | 9.34 | 22.6 |
| Mean | | 7.36 | 9.34 | 22.60 |
| std | | 0.01 | 0.00 | 0.00 |
| Grand Mean | | 7.36 | 9.33 | 22.61 |
| Std | | 0.01 | 0.01 | 0.02 |
| Minimum | | 7.32 | 9.31 | 22.60 |
| Maximum | | 7.38 | 9.34 | 22.70 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

Data Forms: "Water Quality - Temperature (°C) Measurements, "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements"

Item Number 1
Page 2 of 2

File Folder: 11b

| | | | | |
|---|---|---|---|---|
| Study Number: AEH-12-PSEUDO-04 | | Action | Date | Initials |
| Electronic Lab Notebook (pages 21 to 23) | | Created...... | 19-Oct-13 | KLW |
| Data Source: File Folder: 11b | | Revised...... | 10-Dec-14 | KLW |
| Forms: | "Water Quality - Temperature (°C) Measurements" | Reviewed... | 10Dec14 | |
| | "Water Quality - pH Measurements" | Certified... | 12/10/14 | |
| | "Water Quality - Dissolved Oxygen (mg/L) Measurements" | | | |
| File Name: | I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Shawano Water Chem (Whole Tank) 11-26-14.xlsx]Exposure Water Chem | | | |

**Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

Data Explanation:
Water chemistry measurements (dissolved oxygen, pH and temperature) were observed for all test tanks at 0, 3, 6, 9 and 12 h after dosing.
NOTE: 0 h measurements were observed from 30 minutes to 1 h after dosing.

Data anomalies and deviations:
NONE

File Folder: 11b

Item Number 2
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 21 - 23)
Data Source: File Folder: 11b
Forms: "Water Quality - Temperature (°C) Measurements"
"Water Quality - pH Measurements"
"Water Quality - Dissolved Oxygen (mg/L) Measurements"

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

## Exposure Water Chemistry

| Treatment Level (mg/L) | Test Tank ID | 0 hour DO (mg/L) | 0 hour pH[1] | 0 hour Temp. (°C) | 3 Hour DO (mg/L) | 3 Hour pH[1] | 3 Hour Temp. (°C) | 6 Hour DO (mg/L) | 6 Hour pH[1] | 6 Hour Temp. (°C) | 9 Hour DO (mg/L) | 9 Hour pH[1] | 9 Hour Temp. (°C) | 12 Hour DO (mg/L) | 12 Hour pH[1] | 12 Hour Temp. (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 7.21 | 9.27 | 22.0 | 7.02 | 9.24 | 22.3 | 6.78 | 9.14 | 22.6 | 6.61 | 9.12 | 22.7 | 6.30 | 9.02 | 22.6 |
| | 4 | 7.34 | 9.28 | 22.0 | 7.10 | 9.23 | 22.0 | 6.93 | 9.15 | 22.0 | 6.65 | 9.16 | 22.1 | 6.52 | 9.06 | 22.0 |
| | 7 | 7.18 | 9.28 | 21.9 | 7.08 | 9.23 | 21.8 | 6.83 | 9.15 | 21.8 | 6.63 | 9.15 | 21.7 | 6.48 | 9.08 | 21.5 |
| Mean | | 7.24 | 9.28 | 22.0 | 7.07 | 9.23 | 22.0 | 6.85 | 9.15 | 22.1 | 6.63 | 9.14 | 22.2 | 6.43 | 9.05 | 22.0 |
| Std | | 0.07 | 0.00 | 0.05 | 0.03 | 0.00 | 0.21 | 0.06 | 0.00 | 0.34 | 0.02 | 0.02 | 0.41 | 0.10 | 0.02 | 0.45 |
| 50 | 2 | 7.26 | 9.22 | 22.0 | 7.11 | 9.12 | 22.0 | 6.67 | 9.08 | 22.2 | 6.01 | 9.02 | 22.1 | 4.65 | 8.83 | 22.1 |
| | 5 | 7.27 | 9.22 | 21.8 | 6.92 | 9.14 | 21.8 | 6.58 | 9.07 | 21.7 | 5.98 | 9.02 | 21.7 | 4.69 | 8.85 | 21.6 |
| | 8 | 7.18 | 9.22 | 21.9 | 6.89 | 9.11 | 21.9 | 6.47 | 9.06 | 21.8 | 5.59 | 8.99 | 21.8 | 3.31 | 8.73 | 21.7 |
| Mean | | 7.24 | 9.22 | 21.9 | 6.97 | 9.12 | 21.9 | 6.57 | 9.07 | 21.9 | 5.86 | 9.01 | 21.9 | 4.22 | 8.81 | 21.8 |
| Std | | 0.04 | 0.00 | 0.08 | 0.10 | 0.01 | 0.08 | 0.08 | 0.01 | 0.22 | 0.19 | 0.01 | 0.17 | 0.64 | 0.05 | 0.22 |
| 100 | 3 | 7.16 | 9.14 | 22.0 | 6.97 | 9.08 | 22.0 | 6.50 | 8.98 | 22.0 | 6.03 | 8.91 | 22.0 | 5.07 | 8.73 | 21.9 |
| | 6 | 7.29 | 9.14 | 21.9 | 7.04 | 9.07 | 21.8 | 6.68 | 9.00 | 21.8 | 6.25 | 8.93 | 21.8 | 5.29 | 8.68 | 21.7 |
| | 9 | 7.31 | 9.16 | 21.9 | 7.02 | 9.08 | 21.9 | 6.50 | 8.97 | 22.1 | 5.80 | 8.87 | 22.1 | 4.02 | 8.62 | 21.7 |
| Mean | | 7.25 | 9.15 | 21.9 | 7.01 | 9.08 | 21.9 | 6.56 | 8.98 | 22.0 | 6.03 | 8.90 | 22.0 | 4.79 | 8.68 | 21.8 |
| Std | | 0.07 | 0.01 | 0.05 | 0.03 | 0.00 | 0.08 | 0.08 | 0.01 | 0.12 | 0.18 | 0.02 | 0.12 | 0.55 | 0.04 | 0.09 |
| Grand Mean | | 7.24 | 9.22 | 21.9 | 7.02 | 9.15 | 21.9 | 6.66 | 9.07 | 22.0 | 6.17 | 9.03 | 22.0 | 5.15 | 8.88 | 21.9 |
| (STD) | | 0.01 | 0.05 | 0.03 | 0.04 | 0.07 | 0.06 | 0.13 | 0.07 | 0.10 | 0.33 | 0.10 | 0.12 | 0.94 | 0.16 | 0.12 |
| Minimum | | 7.16 | 9.14 | 21.8 | 6.89 | 9.07 | 21.8 | 6.47 | 8.97 | 21.7 | 5.59 | 8.87 | 21.7 | 3.31 | 8.62 | 21.5 |
| Maximum | | 7.34 | 9.28 | 22.0 | 7.11 | 9.24 | 22.3 | 6.93 | 9.15 | 22.6 | 6.65 | 9.16 | 22.7 | 6.52 | 9.08 | 22.6 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

File Folder: 11b

Item Number 2
Page 2 of 2

| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (page 23)<br>TAN Data Source: LTRMP Report (File Folder 17)<br>pH and Temperature Data Source: Water Quality Forms (File Folder 11b) | Action | Date | Initials |
|---|---|---|---|
| | Created...... | 19-Oct-13 | KLW KW |
| | Revised...... | 10-Dec-14 | KLW KW |
| | Reviewed... | 10DEC14 | KW |
| | Certified...... | 12/10/14 | JAL |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Shawano Water Chem (Whole Tank) 11-26-14.xlsx

**Exposure Un-ionized Ammonia**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

Data Explanation:
1) Water samples were collected at 12 h from each exposure tank. Samples were 0.45 µm filtered, acidified with sulfuric acid, and analyzed by the 4500-$NH_3$ G. Automated Phenate Method (Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005) on a Technicon Autoanalyzer II by the UMESC water quality laboratory.
2) The un-ionized ammonia fractions were calculated using the sample pH and temperature according to the formula in Emerson et al. (1975).

Data Anomalies and Deviations:
1) Water samples were not collected at 6 and 9 h from the exposure tanks for un-ionized ammonia analysis. See Deviation #3 for further clarification.

File Folder: 11b

Item Number 3
Page 1 of 3

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 23)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 11b)

Test Article: MB 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

## Exposure Un-ionized Ammonia

| Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|
| | 1 | 9.02 | 22.6 | 0.133 | 0.334 | 0.044 |
| 0 | 4 | 9.06 | 22.0 | 0.126 | 0.344 | 0.043 |
| | 7 | 9.08 | 21.5 | 0.123 | 0.347 | 0.043 |
| Mean | | 9.05 | 22.0 | 0.127 | 0.342 | 0.043 |
| Std | | 0.02 | 0.4 | 0.004 | 0.006 | 0.001 |
| | 2 | 8.83 | 22.1 | 0.188 | 0.238 | 0.045 |
| 50 | 5 | 8.85 | 21.6 | 0.176 | 0.239 | 0.042 |
| | 8 | 8.73 | 21.7 | 0.198 | 0.194 | 0.038 |
| Mean | | 8.81 | 21.8 | 0.187 | 0.224 | 0.042 |
| Std | | 0.05 | 0.2 | 0.009 | 0.021 | 0.003 |
| | 3 | 8.73 | 21.9 | 0.226 | 0.196 | 0.044 |
| 100 | 6 | 8.68 | 21.7 | 0.223 | 0.177 | 0.039 |
| | 9 | 8.62 | 21.7 | 0.241 | 0.157 | 0.038 |
| Mean | | 8.68 | 21.8 | 0.230 | 0.177 | 0.041 |
| Std | | 0.04 | 0.1 | 0.008 | 0.016 | 0.003 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction ($f$) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15+T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018+\left(\frac{2729.92}{(273.15+T_c)}\right)\right)-pH}\right]+1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia x= f * TAN (mg/L)

Item Number 3
Page 2 of 3

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 23)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 11b)

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

## Exposure Un-ionized Ammonia

| Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|
| | 1 | 9.02 | 22.6 | 0.133 | x=1/(10^(0.09018+(2729.92/(273.15+F11))-E11)+1) | x=H11*G11 |
| 0 | 4 | 9.06 | 22.0 | 0.126 | x=1/(10^(0.09018+(2729.92/(273.15+F12))-E12)+1) | x=H12*G12 |
| | 7 | 9.08 | 21.5 | 0.123 | x=1/(10^(0.09018+(2729.92/(273.15+F13))-E13)+1) | x=H13*G13 |
| Mean | | x=(LOG10((10^E11+10^E12+10^E13)/3)) | x=AVERAGE(F11:F13) | x=AVERAGE(G11:G13) | x=AVERAGE(H11:H13) | x=AVERAGE(I11:I13) |
| Std | | x=STDEV.P(E11:E13) | x=STDEV.P(F11:F13) | x=STDEV.P(G11:G13) | x=STDEV.P(H11:H13) | x=STDEV.P(I11:I13) |
| | 2 | 8.83 | 22.1 | 0.188 | x=1/(10^(0.09018+(2729.92/(273.15+F16))-E16)+1) | x=H16*G16 |
| 50 | 5 | 8.85 | 21.6 | 0.176 | x=1/(10^(0.09018+(2729.92/(273.15+F17))-E17)+1) | x=H17*G17 |
| | 8 | 8.73 | 21.7 | 0.198 | x=1/(10^(0.09018+(2729.92/(273.15+F18))-E18)+1) | x=H18*G18 |
| Mean | | x=(LOG10((10^E16+10^E17+10^E18)/3)) | x=AVERAGE(F16:F18) | x=AVERAGE(G16:G18) | x=AVERAGE(H16:H18) | x=AVERAGE(I16:I18) |
| Std | | x=STDEV.P(E16:E18) | x=STDEV.P(F16:F18) | x=STDEV.P(G16:G18) | x=STDEV.P(H16:H18) | x=STDEV.P(I16:I18) |
| | 3 | 8.73 | 21.9 | 0.226 | x=1/(10^(0.09018+(2729.92/(273.15+F21))-E21)+1) | x=H21*G21 |
| 100 | 6 | 8.68 | 21.7 | 0.223 | x=1/(10^(0.09018+(2729.92/(273.15+F22))-E22)+1) | x=H22*G22 |
| | 9 | 8.62 | 21.7 | 0.241 | x=1/(10^(0.09018+(2729.92/(273.15+F23))-E23)+1) | x=H23*G23 |
| Mean | | x=(LOG10((10^E21+10^E22+10^E23)/3)) | x=AVERAGE(F21:F23) | x=AVERAGE(G21:G23) | x=AVERAGE(H21:H23) | x=AVERAGE(I21:I23) |
| Std | | x=STDEV.P(E21:E23) | x=STDEV.P(F21:F23) | x=STDEV.P(G21:G23) | x=STDEV.P(H21:H23) | x=STDEV.P(I21:I23) |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction($f$) is calculated based on the following formula (Emerson et al. 1975): $f=\frac{1}{(10^{pKa-pH})+1}$; $pKa=0.09018+\frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15+T_c)}$; The final calculation used is then: $f=\frac{1}{\left\{\left[10^{\left(0.09018+\left(\frac{2729.92}{(273.15+T_c)}\right)\right)-pH}\right]+1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia x= f * TAN (mg/L)

**File Folder:** 11b

Item Number 3
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| | | | |
|---|---|---|---|
| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
| Electronic Lab Notebook (page 14) | Created...... | 19-Oct-13 | KLW KLW |
| Data Source: File Folder: 12b | Revised...... | 10-Dec-14 | KLW KLW |
| Forms: See form names as stated below | Reviewed... | 10Dec14 | KW |
| | Certified... | 12/10/14 | JA |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Carlos Water Chem (Bottom Injection) 11-26-14.xlsx]Pre-Exposure Water Chem

**Pre-Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

Data Explanation:
Forms titled "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation": Conductivity, hardness, and alkalinity were measured prior to dosing. Samples were collected from both headboxes used to fill individual tanks. Samples were analyzed in triplicate.

Forms titled "Water Quality - Temperature (°C) Measurements", "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements": Temperature, pH and dissolved oxygen levels were measured prior to dosing. Measurements were observed in each exposure tank.

Data anomalies and deviations:
NONE

File Folder: 12b

Item Number 1
Page 1 of 2

Study Number: AEH 12 PSEUDO 04
Electronic Lab Notebook (page 14)
Data Source: File Folder: 12b
Forms: See table footnotes

Test Article: MBI 401 SDP [*Pf*-CL145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

## Pre-Exposure Water Chemistry

| Headbox ID | Replicate | Conductivity (µS) | Hardness (mg/L of $CaCO_3$) | Alkalinity (mg/L of $CaCO_3$) |
|---|---|---|---|---|
| 1 | 1 | 362 | 176 | 164 |
| | 2 | 359 | 178 | 164 |
| | 3 | 360 | 178 | 164 |
| 2 | 1 | 365 | 178 | 164 |
| | 2 | 368 | 176 | 164 |
| | 3 | 362 | 178 | 163 |
| Mean | | 363 | 177 | 164 |
| (STD) | | (3) | (1) | (0) |
| Minimum | | 359 | 176 | 163 |
| Maximum | | 368 | 178 | 164 |

Data Forms: "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation"

| Treatment Level | Test Tank ID | DO (mg/L) | pH[1] | Temp. (°C) |
|---|---|---|---|---|
| 0 | 3 | 8.42 | 8.70 | 21.2 |
| | 6 | 8.40 | 8.70 | 21.2 |
| | 7 | 8.43 | 8.70 | 21.2 |
| Mean | | 8.42 | 8.70 | 21.20 |
| Std | | 0.01 | 0.00 | 0.00 |
| 50 | 1 | 8.39 | 8.70 | 21.2 |
| | 4 | 8.43 | 8.70 | 21.2 |
| | 8 | 8.39 | 8.70 | 21.1 |
| Mean | | 8.40 | 8.70 | 21.17 |
| Std | | 0.02 | 0.00 | 0.05 |
| 100 | 2 | 8.43 | 8.70 | 21.2 |
| | 5 | 8.36 | 8.69 | 21.2 |
| | 9 | 8.40 | 8.69 | 21.2 |
| Mean | | 8.40 | 8.69 | 21.20 |
| Std | | 0.03 | 0.00 | 0.00 |
| Grand Mean | | 8.41 | 8.70 | 21.19 |
| Std | | 0.01 | 0.00 | 0.02 |
| Minimum | | 8.36 | 8.69 | 21.10 |
| Maximum | | 8.43 | 8.70 | 21.20 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

Data Forms: "Water Quality - Temperature (°C) Measurements, "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements"

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File Folder. 12b

| Stucy Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 15 to 16) | Created...... | 19-Oct-13 | KLW |
| Data Source: File Folder: 12b | Revised...... | 10-Dec-14 | KLW |
| Forms: "Water Quality - Temperature (°C) Measurements" | Reviewed... | 10DEC14 | |
| "Water Quality - pH Measurements" | Certified..... | 12/15/14 | |
| "Water Quality - Dissolved Oxygen (mg/L) Measurements" | | | |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Carlos Water Chem (Bottom Injection) 11-26-14.xlsx]Exposure Water Chem | | | |

**Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

Data Explanation:
Water chemistry measurements (dissolved oxygen, pH and temperature) were observed for all test tanks at 0, 3, 6, 9 and 12 h after dosing.
NOTE: 0 h measurements were observed from 30 minutes to 1 h after dosing.

Data anomalies and deviations:
NONE

File Folder: 12b

Item Number 2
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 15 to 16)
Data Source: File Folder: 12b
Forms: "Water Quality - Temperature (°C) Measurements"
"Water Quality - pH Measurements"
"Water Quality - Dissolved Oxygen (mg/L) Measurements"

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

## Exposure Water Chemistry

| Treatment Level (mg/L) | Test Tank ID | 0 hour | | | 3 Hour | | | 6 Hour | | | 9 Hour | | | 12 Hour | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) |
| 0 | 3 | 7.95 | 8.52 | 21.3 | 7.94 | 8.51 | 21.2 | 7.91 | 8.47 | 21.6 | 7.77 | 8.38 | 21.6 | 7.72 | 8.55 | 21.1 |
| | 6 | 7.98 | 8.57 | 21.3 | 7.98 | 8.54 | 21.2 | 7.94 | 8.49 | 21.5 | 7.86 | 8.39 | 21.3 | 7.80 | 8.57 | 21.0 |
| | 7 | 7.99 | 8.59 | 21.3 | 7.98 | 8.55 | 21.2 | 7.99 | 8.50 | 21.3 | 7.88 | 8.39 | 21.2 | 7.84 | 8.58 | 20.9 |
| Mean | | 7.97 | 8.56 | 21.30 | 7.97 | 8.53 | 21.20 | 7.95 | 8.49 | 21.47 | 7.84 | 8.39 | 21.37 | 7.79 | 8.57 | 21.00 |
| STD | | 0.02 | 0.03 | 0.00 | 0.02 | 0.02 | 0.00 | 0.03 | 0.01 | 0.12 | 0.05 | 0.00 | 0.17 | 0.05 | 0.01 | 0.08 |
| 50 | 1 | 7.94 | 8.60 | 21.3 | 7.95 | 8.55 | 21.2 | 7.79 | 8.48 | 21.8 | 7.80 | 8.38 | 21.7 | 6.83 | 8.12 | 21.3 |
| | 4 | 8.00 | 8.61 | 21.3 | 7.99 | 8.55 | 21.3 | 7.80 | 8.48 | 21.9 | 7.80 | 8.39 | 21.8 | 7.21 | 8.19 | 21.3 |
| | 8 | 7.97 | 8.60 | 21.3 | 7.96 | 8.55 | 21.2 | 7.90 | 8.48 | 21.3 | 7.83 | 8.41 | 21.2 | 7.45 | 8.20 | 20.8 |
| Mean | | 7.97 | 8.60 | 21.30 | 7.97 | 8.55 | 21.23 | 7.83 | 8.48 | 21.67 | 7.81 | 8.39 | 21.57 | 7.16 | 8.17 | 21.13 |
| STD | | 0.02 | 0.00 | 0.00 | 0.02 | 0.00 | 0.05 | 0.05 | 0.00 | 0.26 | 0.01 | 0.01 | 0.26 | 0.26 | 0.04 | 0.24 |
| 100 | 2 | 8.00 | 8.60 | 21.2 | 7.98 | 8.55 | 21.2 | 7.93 | 8.48 | 21.7 | 7.87 | 8.41 | 21.6 | 7.44 | 7.18 | 21.2 |
| | 5 | 7.80 | 8.58 | 21.4 | 7.92 | 8.54 | 21.3 | 7.89 | 8.46 | 21.5 | 7.74 | 8.39 | 21.4 | 7.13 | 7.39 | 21.1 |
| | 9 | 7.90 | 8.57 | 21.3 | 7.96 | 8.52 | 21.3 | 7.91 | 8.44 | 21.5 | 7.82 | 8.40 | 21.4 | 7.14 | 7.34 | 21.0 |
| Mean | | 7.90 | 8.58 | 21.30 | 7.95 | 8.54 | 21.27 | 7.91 | 8.46 | 21.57 | 7.81 | 8.40 | 21.47 | 7.24 | 7.31 | 21.10 |
| STD | | 0.08 | 0.01 | 0.08 | 0.02 | 0.01 | 0.05 | 0.02 | 0.02 | 0.09 | 0.05 | 0.01 | 0.09 | 0.14 | 0.09 | 0.08 |
| Grand Mean | | 7.95 | 8.58 | 21.3 | 7.96 | 8.54 | 21.2 | 7.90 | 8.48 | 21.6 | 7.82 | 8.39 | 21.5 | 7.40 | 8.25 | 21.1 |
| STD | | 0.03 | 0.02 | 0.00 | 0.01 | 0.01 | 0.03 | 0.05 | 0.01 | 0.08 | 0.01 | 0.01 | 0.08 | 0.28 | 0.52 | 0.06 |
| Minimum | | 7.80 | 8.52 | 21.2 | 7.92 | 8.51 | 21.2 | 7.79 | 8.44 | 21.3 | 7.74 | 8.38 | 21.2 | 6.83 | 7.18 | 20.8 |
| Maximum | | 8.00 | 8.61 | 21.4 | 7.99 | 8.55 | 21.3 | 7.99 | 8.50 | 21.9 | 7.88 | 8.41 | 21.8 | 7.84 | 8.58 | 21.3 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

File Folder: 12b

Item Number 2
Page 2 of 2

| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (page 16)<br>TAN Data Source: LTRMP Report (File Folder 17)<br>pH and Temperature Data Source: Water Quality Forms (File Folder 12b) | Action | Date | Initials |
|---|---|---|---|
| | Created...... | 19-Oct-13 | KLW KW |
| | Revised...... | 10-Dec-14 | KLW KW |
| | Reviewed... | 10DEC14 | KW |
| | Certified...... | 12/10/14 | JA |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Carlos Water Chem (Bottom Injection) 11-26-14.xlsx]Ammonia Data

**Exposure Un-Ionized Ammonia**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

Data Explanation:
1) Water samples were collected at 12 h from each exposure tank. Samples were 0.45 µm filtered, acidified with sulfuric acid, and analyzed by the 4500-$NH_3$ G. Automated Phenate Method (Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005) on a Technicon Autoanalyzer II by the UMESC water quality laboratory.
2) The un-ionized ammonia fractions were calculated using the sample pH and temperature according to the formula in Emerson et al. (1975).

Data Anomalies and Deviations:
NONE

File Folder: 12b

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Page 1 of 3

Study Number: AEH-12-PSEUDO-03
Electronic Lab Notebook (page 16)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 12B)

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

**Exposure Un-ionized Ammonia**

| Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|
| | 3 | 8.55 | 21.1 | 0.232 | 0.132 | 0.031 |
| 0 | 6 | 8.57 | 21.0 | 0.207 | 0.137 | 0.028 |
| | 7 | 8.58 | 20.9 | 0.210 | 0.138 | 0.029 |
| Mean | | 8.57 | 21.0 | 0.216 | 0.136 | 0.029 |
| std | | 0.01 | 0.08 | 0.01 | 0.00 | 0.00 |
| | 1 | 8.12 | 21.3 | 0.403 | 0.054 | 0.022 |
| 50 | 4 | 8.19 | 21.3 | 0.415 | 0.063 | 0.026 |
| | 8 | 8.20 | 20.8 | 0.385 | 0.062 | 0.024 |
| Mean | | 8.17 | 21.1 | 0.401 | 0.060 | 0.024 |
| std | | 0.04 | 0.24 | 0.01 | 0.00 | 0.00 |
| | 2 | 7.18 | 21.2 | 1.486 | 0.006 | 0.010 |
| 100 | 5 | 7.39 | 21.1 | 1.123 | 0.010 | 0.012 |
| | 9 | 7.34 | 21.0 | 1.504 | 0.009 | 0.014 |
| Mean | | 7.31 | 21.1 | 1.371 | 0.009 | 0.012 |
| std | | 0.09 | 0.08 | 0.18 | 0.00 | 0.00 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction($f$) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15+T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018+\left(\frac{2729.92}{(273.15+T_c)}\right)\right)-pH}\right]+1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia = $f$ * TAN (mg/L)

Item Number 3
Page 2 of 3

Study Number: AEH-12-PSEUDO-03
Electronic Lab Notebook (page 16)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 12B)

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

## Exposure Un-ionized Ammonia

| Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|
| | 3 | 8.55 | 21.1 | 0.232 | x=1/(10^(0.09018+(2729.92/(273.15+F11))-E11)+1) | x=H11*G11 |
| 0 | 6 | 8.57 | 21.0 | 0.207 | x=1/(10^(0.09018+(2729.92/(273.15+F12))-E12)+1) | x=H12*G12 |
| | 7 | 8.58 | 20.9 | 0.210 | x=1/(10^(0.09018+(2729.92/(273.15+F13))-E13)+1) | x=H13*G13 |
| Mean | | x=(LOG10((10^E11+10^E12+10^E13)/3)) | x=AVERAGE(F11:F13) | x=AVERAGE(G11:G13) | x=AVERAGE(H11:H13) | x=AVERAGE(I11:I13) |
| std | | x=STDEV.P(E11:E13) | x=STDEV.P(F11:F13) | x=STDEV.P(G11:G13) | x=STDEV.P(H11:H13) | x=STDEV.P(I11:I13) |
| | 1 | 8.12 | 21.3 | 0.403 | x=1/(10^(0.09018+(2729.92/(273.15+F16))-E16)+1) | x=H16*G16 |
| 50 | 4 | 8.19 | 21.3 | 0.415 | x=1/(10^(0.09018+(2729.92/(273.15+F17))-E17)+1) | x=H17*G17 |
| | 8 | 8.20 | 20.8 | 0.385 | x=1/(10^(0.09018+(2729.92/(273.15+F18))-E18)+1) | x=H18*G18 |
| Mean | | x=(LOG10((10^E16+10^E17+10^E18)/3)) | x=AVERAGE(F16:F18) | x=AVERAGE(G16:G18) | x=AVERAGE(H16:H18) | x=AVERAGE(I16:I18) |
| std | | x=STDEV.P(E16:E18) | x=STDEV.P(F16:F18) | x=STDEV.P(G16:G18) | x=STDEV.P(H16:H18) | x=STDEV.P(I16:I18) |
| | 2 | 7.18 | 21.2 | 1.486 | x=1/(10^(0.09018+(2729.92/(273.15+F21))-E21)+1) | x=H21*G21 |
| 100 | 5 | 7.39 | 21.1 | 1.123 | x=1/(10^(0.09018+(2729.92/(273.15+F22))-E22)+1) | x=H22*G22 |
| | 9 | 7.34 | 21.0 | 1.504 | x=1/(10^(0.09018+(2729.92/(273.15+F23))-E23)+1) | x=H23*G23 |
| Mean | | x=(LOG10((10^E21+10^E22+10^E23)/3)) | x=AVERAGE(F21:F23) | x=AVERAGE(G21:G23) | x=AVERAGE(H21:H23) | x=AVERAGE(I21:I23) |
| std | | x=STDEV.P(E21:E23) | x=STDEV.P(F21:F23) | x=STDEV.P(G21:G23) | x=STDEV.P(H21:H23) | x=STDEV.P(I21:I23) |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction ($f$) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15 - T_c)}$; The final calculation used is then: $f = \frac{1}{\left\{\left[10^{\left(0.09018+\left(\frac{2729.92}{(273.15+T_c)}\right)\right)-pH}\right]+1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia x= f * TAN (mg/L)

File Folder: 12B

Item Number 3

Page 3 of 3

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (page 25) | Created...... | 19-Oct-13 | KLW KW |
| Data Source: File Folder: 14b | Revised...... | 10-Dec-14 | KLW KW |
| Forms: See form names as stated below | Reviewed... | 10DEC14 | KW |
| | Certified... | 12/10/14 | JR |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Shawano Water Chem (Bottom Injection) 11-26-14.xlsx]Pre-Exposure Water Chem | | | |

**Pre-Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

Data Explanation:

Forms titled "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation": Conductivity, hardness, and alkalinity were measured prior to dosing. Samples were collected from both headboxes used to fill individual tanks. Samples were analyzed in triplicate.

Forms titled "Water Quality - Temperature (°C) Measurements", "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements": Temperature, pH and dissolved oxygen levels were measured prior to dosing. Measurements were observed in each exposure tank.

Data anomalies and deviations:

NONE

File Folder: 14b

Item Number 1
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 25)
Data Source: File Folder: 14b
Forms: See table footnotes

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

## Pre-Exposure Water Chemistry

| Headbox ID | Replicate | Conductivity (µS) | Hardness (mg/L of $CaCO_3$) | Alkalinity (mg/L of $CaCO_3$) |
|---|---|---|---|---|
| 1 | 1 | 227 | 124 | 112 |
| | 2 | 233 | 124 | 111 |
| | 3 | 231 | 124 | 112 |
| 2 | 1 | 234 | 126 | 112 |
| | 2 | 233 | 126 | 111 |
| | 3 | 230 | 126 | 111 |
| Mean | | 231 | 125 | 112 |
| (STD) | | (2) | (1) | (1) |
| Minimum | | 227 | 124 | 111 |
| Maximum | | 234 | 126 | 112 |

Data Forms: "Conductivity and Hardness - Exposure Initiation" and "Alkalinity - Exposure Initiation"

| Treatment Level | Test Tank ID | DO (mg/L) | pH[1] | Temp. (°C) |
|---|---|---|---|---|
| 0 | 1 | 7.40 | 9.10 | 19.5 |
| | 9 | 7.40 | 9.11 | 19.5 |
| | 8 | 7.42 | 9.12 | 19.5 |
| Mean | | 7.41 | 9.11 | 19.50 |
| std | | 0.01 | 0.01 | 0.00 |
| 50 | 2 | 7.43 | 9.13 | 19.5 |
| | 3 | 7.42 | 9.13 | 19.5 |
| | 7 | 7.43 | 9.14 | 19.5 |
| Mean | | 7.43 | 9.13 | 19.50 |
| std | | 0.00 | 0.00 | 0.00 |
| 100 | 4 | 7.44 | 9.14 | 19.5 |
| | 6 | 7.44 | 9.14 | 19.5 |
| | 5 | 7.45 | 9.13 | 19.5 |
| Mean | | 7.44 | 9.14 | 19.50 |
| std | | 0.00 | 0.00 | 0.00 |
| Grand Mean | | 7.43 | 9.13 | 19.50 |
| Std | | 0.01 | 0.01 | 0.00 |
| Minimum | | 7.40 | 9.10 | 19.50 |
| Maximum | | 7.45 | 9.14 | 19.50 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

Data Forms: "Water Quality - Temperature (°C) Measurements, "Water Quality - pH Measurements" and "Water Quality - Dissolved Oxygen (mg/L) Measurements"

Item Number 1
Page 2 of 2

File Folder: 14b

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 26 - 27) | Created..... | 19-Oct-13 | KLW |
| Data Source: File Folder: 14b | Revised..... | 10-Dec-14 | KLW |
| Forms: "Water Quality - Temperature (°C) Measurements" | Reviewed... | 10 DEC 14 | |
| "Water Quality - pH Measurements" | Certified... | 12/10/14 | |
| "Water Quality - Dissolved Oxygen (mg/L) Measurements" | | | |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Shawano Water Chem (Bottom Injection) 11-26-14.xlsx]Exposure Water Chem | | | |

**Exposure Water Chemistry**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

Data Explanation:
Water chemistry measurements (dissolved oxygen, pH and temperature) were observed for all test tanks at 0, 3, 6, 9 and 12 h after dosing.
NOTE: 0 h measurements were observed from 30 minutes to 1 h after dosing.

Data anomalies and deviations:
NONE

File Folder: 14b

Item Number 2
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 26 to 27)
Data Source: File Folder: 14b
Forms: "Water Quality - Temperature (°C) Measurements"
"Water Quality - pH Measurements"
"Water Quality Dissolved Oxygen (mg/L) Measurements"

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

## Exposure Water Chemistry

| Treatment Level (mg/L) | Test Tanks ID | 0 hour | | | 3 Hour | | | 6 Hour | | | 9 Hour | | | 12 Hour | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) | DO (mg/L) | pH[1] | Temp. (°C) |
| | 1 | 7.25 | 9.05 | 18.3 | 7.06 | 9.02 | 18.8 | 7.01 | 8.91 | 19.0 | 6.98 | 8.64 | 18.8 | 6.61 | 8.92 | 18.5 |
| 0 | 9 | 7.27 | 9.06 | 18.0 | 7.20 | 9.03 | 18.2 | 7.03 | 8.92 | 18.4 | 7.03 | 8.68 | 18.4 | 6.65 | 8.94 | 18.1 |
| | 8 | 7.25 | 9.06 | 18.2 | 7.20 | 9.03 | 18.2 | 7.01 | 8.94 | 18.4 | 6.93 | 8.68 | 18.3 | 6.62 | 8.92 | 18.1 |
| Mean | | 7.26 | 9.06 | 18.2 | 7.15 | 9.03 | 18.4 | 7.02 | 8.92 | 18.6 | 6.98 | 8.67 | 18.5 | 6.63 | 8.93 | 18.2 |
| std | | 0.01 | 0.00 | 0.12 | 0.07 | 0.00 | 0.28 | 0.01 | 0.01 | 0.28 | 0.04 | 0.02 | 0.22 | 0.02 | 0.01 | 0.19 |
| | 2 | 7.27 | 9.02 | 18.2 | 7.24 | 9.02 | 18.5 | 7.26 | 8.93 | 18.7 | 7.19 | 8.71 | 18.6 | 4.55 | 8.69 | 18.3 |
| 50 | 3 | 7.21 | 9.04 | 18.3 | 7.24 | 9.04 | 18.4 | 7.31 | 8.95 | 18.5 | 7.20 | 8.71 | 18.4 | 4.74 | 8.88 | 18.3 |
| | 7 | 7.19 | 9.04 | 18.2 | 7.21 | 9.04 | 18.2 | 7.27 | 8.95 | 18.2 | 7.04 | 8.69 | 18.1 | 6.05 | 8.84 | 18.0 |
| Mean | | 7.22 | 9.03 | 18.2 | 7.23 | 9.03 | 18.4 | 7.28 | 8.94 | 18.5 | 7.14 | 8.70 | 18.4 | 5.11 | 8.81 | 18.2 |
| std | | 0.03 | 0.01 | 0.05 | 0.01 | 0.01 | 0.12 | 0.02 | 0.01 | 0.21 | 0.07 | 0.01 | 0.21 | 0.67 | 0.08 | 0.14 |
| | 4 | 7.16 | 8.99 | 18.1 | 7.27 | 9.03 | 18.2 | 7.34 | 8.95 | 18.3 | 7.24 | 8.71 | 18.3 | 6.65 | 8.68 | 18.0 |
| 100 | 6 | 7.14 | 9.01 | 18.1 | 7.21 | 9.03 | 18.2 | 7.27 | 8.96 | 18.1 | 7.25 | 8.69 | 18.2 | 6.15 | 8.58 | 17.8 |
| | 5 | 7.22 | 9.01 | 17.9 | 7.22 | 9.03 | 18.0 | 7.27 | 8.96 | 18.2 | 7.22 | 8.71 | 18.0 | 5.83 | 8.59 | 18.0 |
| Mean | | 7.17 | 9.00 | 18.0 | 7.23 | 9.03 | 18.1 | 7.29 | 8.96 | 18.2 | 7.24 | 8.70 | 18.2 | 6.21 | 8.62 | 17.9 |
| std | | 0.03 | 0.01 | 0.09 | 0.03 | 0.00 | 0.09 | 0.03 | 0.00 | 0.08 | 0.01 | 0.01 | 0.12 | 0.34 | 0.04 | 0.09 |
| Grand Mean | | 7.22 | 9.03 | 18.1 | 7.21 | 9.03 | 18.3 | 7.20 | 8.94 | 18.4 | 7.12 | 8.69 | 18.3 | 5.98 | 8.80 | 18.1 |
| std | | 0.03 | 0.02 | 0.08 | 0.04 | 0.00 | 0.12 | 0.13 | 0.01 | 0.17 | 0.11 | 0.02 | 0.14 | 0.64 | 0.13 | 0.13 |
| Minimum | | 7.14 | 8.99 | 17.9 | 7.06 | 9.02 | 18.0 | 7.01 | 8.91 | 18.1 | 6.93 | 8.64 | 18.0 | 4.55 | 8.58 | 17.8 |
| Maximum | | 7.27 | 9.06 | 18.3 | 7.27 | 9.04 | 18.8 | 7.34 | 8.96 | 19.0 | 7.25 | 8.71 | 18.8 | 6.65 | 8.94 | 18.5 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

File 14b

Item Number 2
Page 2 of 2

| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (page 27)<br>TAN Data Source: LTRMP Report (File Folder 17)<br>pH and Temperature Data Source: Water Quality Forms (File Folder 14b) | Action | Date | Initials |
|---|---|---|---|
| | Created...... | 19-Oct-13 | KLW KW |
| | Revised...... | 10-Dec-14 | KLW KW |
| | Reviewed... | 10Dec14 | KW |
| | Certified...... | 12/10/14 | JO |

File Name: I:\AEH-12-PSEUDO-04\Data Summaries\Water chem\[Lake Shawano Water Chem (Bottom Injection) 11-26-14

**Exposure Un-ionized Ammonia**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

Data Explanation:
1) Water samples were collected at 12 h from each exposure tank. Samples were 0.45 µm filtered, acidified with sulfuric acid, and analyzed by the 4500-$NH_3$ G. Automated Phenate Method (Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005) on a Technicon Autoanalyzer II by the UMESC water quality laboratory.
2) The un-ionized ammonia fractions were calculated using the sample pH and temperature according to the formula in Emerson et al. (1975).

Data Anomalies and Deviations:
NONE

File Folder: 14b

Item Number 3
Page 1 of 3

## Exposure Un-ionized Ammonia

| Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|
| | 1 | 8.92 | 18.5 | 0.061 | 0.228 | 0.014 |
| 0 | 9 | 8.94 | 18.1 | 0.072 | 0.231 | 0.017 |
| | 8 | 8.92 | 18.1 | 0.056 | 0.223 | 0.012 |
| Mean | | 8.93 | 18.2 | 0.063 | 0.227 | 0.014 |
| std | | 0.01 | 0.19 | 0.007 | 0.003 | 0.002 |
| | 2 | 8.69 | 18.3 | 0.113 | 0.146 | 0.017 |
| 50 | 3 | 8.88 | 18.3 | 0.120 | 0.209 | 0.025 |
| | 7 | 8.84 | 18.0 | 0.094 | 0.191 | 0.018 |
| Mean | | 8.81 | 18.2 | 0.109 | 0.182 | 0.020 |
| std | | 0.08 | 0.14 | 0.011 | 0.027 | 0.004 |
| | 4 | 8.68 | 18.0 | 0.158 | 0.141 | 0.022 |
| 100 | 6 | 8.58 | 17.8 | 0.145 | 0.113 | 0.016 |
| | 5 | 8.59 | 18.0 | 0.163 | 0.117 | 0.019 |
| Mean | | 8.62 | 17.9 | 0.155 | 0.124 | 0.019 |
| std | | 0.04 | 0.09 | 0.008 | 0.012 | 0.002 |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction ($f$) is calculated based on the following formula (Emerson et al. 1975): $f = \frac{1}{(10^{pKa-pH})+1}$; $pKa = 0.09018 + \frac{2729.92}{T_k}$

$T_k = T_c + 273.15$; $pKa = 0.09018 + \frac{2729.92}{(273.15+T_c)}$; The final calculation used is then : $f = \frac{1}{\left\{\left[10^{\left(0.09018+\left(\frac{2729.92}{(273.15+T_C)}\right)\right)-pH}\right]+1\right\}}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia = $f$ * TAN (mg/L)

Item Number 3
Page 2 of 3

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (page 27)
TAN Data Source: LTRMP Report (File Folder 17)
pH and Temperature Data Source: Water Quality Forms (File Folder 14b)

Test Article: MBI 401 SDP [*Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

## Exposure Un-ionized Ammonia

| Treatment Level (mg/L) | Test Tank ID | pH[1] | Temperature (°C) | TAN as $NH_3$-N (mg/L) | Un-ionized Fraction[2] | $NH_3$ (mg/L)[3] |
|---|---|---|---|---|---|---|
| 0 | 1 | 8.92 | 18.5 | 0.061 | x=1/(10^(0.09018+(2729.92/(273.15+D11))-C11)+1) | x=F11*E11 |
| | 9 | 8.94 | 18.1 | 0.072 | x=1/(10^(0.09018+(2729.92/(273.15+D12))-C12)+1) | x=F12*E12 |
| | 8 | 8.92 | 18.1 | 0.056 | x=1/(10^(0.09018+(2729.92/(273.15+D13)) C13)+1) | x=F13*E13 |
| Mean | | x=(LOG10((10^C11+10^C12+10^C13)/3)) | x=AVERAGE(D11:D13) | x=AVERAGE(E11:E13) | x=AVERAGE(F11:F13) | x=AVERAGE(G11:G13) |
| std | | x=STDEV.P(C11:C13) | x=STDEV.P(D11:D13) | x=STDEV.P(E11:E13) | x=STDEV.P(F11:F13) | x=STDEV.P(G11:G13) |
| 50 | 2 | 8.69 | 18.3 | 0.113 | x=1/(10^(0.09018+(2729.92/(273.15+D16))-C16)+1) | x=F16*E16 |
| | 3 | 8.88 | 18.3 | 0.120 | x=1/(10^(0.09018+(2729.92/(273.15+D17))-C17)+1) | x=F17*E17 |
| | 7 | 8.84 | 18.0 | 0.094 | x=1/(10^(0.09018+(2729.92/(273.15+D18))-C18)+1) | x=F18*E18 |
| Mean | | x=(LOG10((10^C16+10^C17+10^C18)/3)) | x=AVERAGE(D16:D18) | x=AVERAGE(E16:E18) | x=AVFRAGF(F16:F18) | x=AVERAGE(G16:G18) |
| std | | x=STDEV.P(C16:C18) | x=STDEV.P(D16:D18) | x=STDEV.P(E16:E18) | x=STDEV.P(F16:F18) | x=STDEV.P(G16:G18) |
| 100 | 4 | 8.68 | 18.0 | 0.158 | x=1/(10^(0.09018+(2729.92/(273.15+D21))-C21)+1) | x=F21*E21 |
| | 6 | 8.58 | 17.8 | 0.145 | x=1/(10^(0.09018+(2729.92/(273.15+D22))-C22)+1) | x=F22*E22 |
| | 5 | 8.59 | 18.0 | 0.163 | x=1/(10^(0.09018+(2729.92/(273.15+D23))-C23)+1) | x=F23*E23 |
| Mean | | x=(LOG10((10^C21+10^C22+10^C23)/3)) | x=AVERAGE(D21:D23) | x=AVERAGE(E21:E23) | x=AVERAGE(F21:F23) | x=AVERAGE(G21:G23) |
| std | | x=STDEV.P(C21:C23) | x=STDEV.P(D21:D23) | x=STDEV.P(E21:E23) | x=STDEV.P(F21:F23) | x=STDEV.P(G21:G23) |

[1] pH means calculated on hydrogen ion concentration; pH standard deviations calculated on pH values

[2] Un-ionized fraction($f$) is calculated based on the following formula (Emerson et al. 1975): $f=\frac{1}{(10^{pKa-pH})+1}$; $pKa=0.09018+\frac{2729.92}{T_k}$

$T_k=T_c+273.15$; $pKa=0.09018+\frac{2729.92}{(273.15+T_c)}$; The final calculation used is then: $f=\frac{1}{\left[10^{\left(0.09018+\left(\frac{2729.92}{(273.15+T_c)}\right)\right)-pH}\right]+1}$

[3] Un-ionized ammonia is calculated based on the following formula: Un-ionized ammonia x= f * TAN (mg/L)

File Folder: 146

Item Number 3
Page 3 of 3

| | | | |
|---|---|---|---|
| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
| Electronic Lab Notebook (pages 13 & 17) | Created...... | 4-Feb-14 | TJS TJS |
| Data Source: File Folder: 17a | Revised...... | 19-Feb-14 | TJS TJS |
| Forms: Onset HOBO Datafile output from HOBO Temperature Loggers (File Folder 18) (I:\AEH-12-PSEUDO-04\Data\Hobos\Lake Carlos HOBO Files) | Reviewed... | 19FEB14 | TJS |
| | Certified... | 2/19/14 | JR |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Lake Temperature Data Loggers.xlsx]Temp Data | | | |

**Temperature Data Loggers (HOBOs)**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15 and 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank and Bottom Injection

Data Explanation:
Upon exposure termination on August 15, 2012 (whole tank) and August 17, 2012 (bottom injection) at Lake Carlos, four wire mesh cages containing test animals were placed into Lake Carlos for a post-exposure holding period. Each cage was fitted with a HOBO temperature logger programmed to record water temperature every six hours (four times per day). Mean daily temperature (°C) and standard deviation were calculated from all daily measurements.

| Data Logger Number | Serial Number | Lake | Treatment Type | Treatment Duration |
|---|---|---|---|---|
| 1 | 10020131 | Carlos | Whole Tank | 6h |
| 2 | 10020140 | Carlos | Whole Tank | 9h |
| 3 | 10020139 | Carlos | Whole Tank | 12h |
| 4 | 10020133 | Carlos | Bottom Injection | 12h |

| Date | Mean Temperature (°C) | (STD) |
|---|---|---|
| 8/17/2012 | 22.61 | 0.61 |
| 8/18/2012 | 22.53 | 0.50 |
| 8/19/2012 | 22.59 | 0.39 |
| 8/20/2012 | 22.38 | 0.32 |
| 8/21/2012 | 22.81 | 0.47 |
| 8/22/2012 | 23.09 | 0.59 |
| 8/23/2012 | 23.37 | 0.47 |
| 8/24/2012 | 24.18 | 0.64 |
| 8/25/2012 | 23.76 | 0.27 |
| 8/26/2012 | 23.93 | 0.53 |
| 8/27/2012 | 24.39 | 0.58 |
| 8/28/2012 | 24.19 | 0.29 |
| 8/29/2012 | 24.07 | 0.48 |
| 8/30/2012 | 24.01 | 0.25 |
| 8/31/2012 | 23.51 | 0.34 |
| 9/1/2012 | 23.27 | 0.36 |
| 9/2/2012 | 23.06 | 0.29 |
| 9/3/2012 | 23.39 | 0.38 |
| 9/4/2012 | 23.39 | 0.25 |
| 9/5/2012 | 23.22 | 0.40 |
| 9/6/2012 | 22.65 | 0.30 |
| 9/7/2012 | 22.16 | 0.37 |
| 9/8/2012 | 21.12 | 0.48 |
| 9/9/2012 | 20.97 | 0.46 |
| 9/10/2012 | 20.84 | 0.22 |
| 9/11/2012 | 20.63 | 0.35 |

Data anomalies and deviations:
Temperature readings from the first and last days of recorder deployment (prior to August 17 and after September 11, 2012 for whole tank; prior to August 19 and after September 11, 2012 for bottom injection) were omitted from data analysis. The temperature recorders may have sampled ambient air temperature during these time points. These entire days will be omitted as diurnal temperature flucuation skew the mean daily temperature. The mean temperature for August 17 and 18, 2012 was calculated using only the three data loggers (#1, 2, and 3) from whole tank treatments.

Item Number 1
Page 1 of 2

File Folder: 17a

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 13 & 17)
Data Source: File Folder: 17a
Forms: HOBO Output in Excel "Lake Carlos 6h whole tank", "Lake Carlos 9h whole tank"
"Lake Carlos 12h whole tank", "Lake Carlos 12h bottom injection"
Location: I:\AEH-12-PSEUDO-04\Data\Hobos\Carlos and Shawano in Excel

Test Article: MBI 401 SDP [Pf-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15 & 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank and Bottom Injection

## Post-Exposure Holding Period Water Temperature

| Date | Data Logger Number 1 | 2 | 3 | 4 | Mean | Date | Data Logger Number 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (GMT-05:00) | Temperature (°C) | | | | (STD) | Time (GMT-05:00) | Temperature (°C) | | | | (STD) |
| 8/17/2012 4:00 | 21.855 | 21.951 | 22.046 | . | | 8/30/2012 4:00 | 23.677 | 23.773 | 23.869 | 23.773 | |
| 8/17/2012 10:00 | 22.429 | 22.621 | 22.238 | . | 22.61 | 8/30/2012 10:00 | 24.351 | 24.158 | 24.255 | 24.062 | 24.01 |
| 8/17/2012 16:00 | 23.484 | 23.484 | 23.773 | . | (0.61) | 8/30/2012 16:00 | 24.255 | 24.158 | 24.255 | 24.448 | (0.25) |
| 8/17/2012 22:00 | 22.429 | 22.525 | 22.525 | . | | 8/30/2012 22:00 | 23.677 | 23.773 | 23.773 | 23.966 | |
| 8/18/2012 4:00 | 21.951 | 21.951 | 22.142 | . | | 8/31/2012 4:00 | 23.196 | 23.196 | 23.292 | 23.581 | |
| 8/18/2012 10:00 | 22.621 | 22.429 | 22.238 | . | 22.53 | 8/31/2012 10:00 | 23.966 | 23.773 | 23.388 | 22.908 | 23.51 |
| 8/18/2012 16:00 | 23.292 | 22.908 | 23.677 | . | (0.50) | 8/31/2012 16:00 | 23.869 | 23.773 | 23.869 | 24.158 | (0.34) |
| 8/18/2012 22:00 | 22.333 | 22.429 | 22.429 | . | | 8/31/2012 22:00 | 23.196 | 23.292 | 23.388 | 23.388 | |
| 8/19/2012 4:00 | 21.951 | 22.046 | 22.142 | 22.238 | | 9/1/2012 4:00 | 22.908 | 23.004 | 23.004 | 23.1 | |
| 8/19/2012 10:00 | 22.717 | 22.621 | 22.525 | 22.333 | 22.59 | 9/1/2012 10:00 | 23.581 | 23.196 | 23.196 | 22.908 | 23.27 |
| 8/19/2012 16:00 | 23.1 | 23.1 | 23.196 | 23.196 | (0.39) | 9/1/2012 16:00 | 23.581 | 23.869 | 23.966 | 23.869 | (0.36) |
| 8/19/2012 22:00 | 22.429 | 22.429 | 22.525 | 22.812 | | 9/1/2012 22:00 | 22.908 | 23.004 | 23.1 | 23.196 | |
| 8/20/2012 4:00 | 21.855 | 21.951 | 22.046 | 22.142 | | 9/2/2012 4:00 | 22.621 | 22.717 | 22.717 | 22.812 | |
| 8/20/2012 10:00 | 22.525 | 22.429 | 22.333 | 21.951 | 22.38 | 9/2/2012 10:00 | 23.292 | 23.004 | 23.004 | 22.717 | 23.06 |
| 8/20/2012 16:00 | 22.717 | 22.717 | 23.004 | 22.717 | (0.32) | 9/2/2012 16:00 | 23.388 | 23.388 | 23.581 | 23.388 | (0.29) |
| 8/20/2012 22:00 | 22.333 | 22.429 | 22.429 | 22.525 | | 9/2/2012 22:00 | 22.908 | 23.004 | 23.1 | 23.292 | |
| 8/21/2012 4:00 | 22.238 | 22.333 | 22.429 | 22.429 | | 9/3/2012 4:00 | 22.812 | 22.812 | 22.908 | 22.908 | |
| 8/21/2012 10:00 | 22.812 | 22.525 | 22.429 | 22.429 | 22.81 | 9/3/2012 10:00 | 23.677 | 23.388 | 23.388 | 23.004 | 23.39 |
| 8/21/2012 16:00 | 23.484 | 23.388 | 23.869 | 23.484 | (0.47) | 9/3/2012 16:00 | 23.677 | 23.677 | 23.773 | 24.062 | (0.38) |
| 8/21/2012 22:00 | 22.717 | 22.717 | 22.812 | 22.908 | | 9/3/2012 22:00 | 23.388 | 23.484 | 23.581 | 23.773 | |
| 8/22/2012 4:00 | 22.142 | 22.238 | 22.333 | 22.429 | | 9/4/2012 4:00 | 23.1 | 23.196 | 23.196 | 23.388 | |
| 8/22/2012 10:00 | 23.581 | 23.1 | 23.1 | 22.717 | 23.09 | 9/4/2012 10:00 | 23.484 | 23.292 | 23.196 | 23.1 | 23.39 |
| 8/22/2012 16:00 | 23.869 | 23.869 | 23.966 | 23.869 | (0.59) | 9/4/2012 16:00 | 23.484 | 23.773 | 23.869 | 23.869 | (0.25) |
| 8/22/2012 22:00 | 23.004 | 23.004 | 23.1 | 23.1 | | 9/4/2012 22:00 | 23.196 | 23.292 | 23.292 | 23.484 | |
| 8/23/2012 4:00 | 22.908 | 22.908 | 23.004 | 23.004 | | 9/5/2012 4:00 | 22.812 | 22.812 | 22.908 | 23.1 | |
| 8/23/2012 10:00 | 23.484 | 23.1 | 23.1 | 22.525 | 23.37 | 9/5/2012 10:00 | 23.484 | 23.196 | 23.292 | 23.1 | 23.22 |
| 8/23/2012 16:00 | 24.062 | 23.869 | 24.448 | 23.581 | (0.47) | 9/5/2012 16:00 | 23.484 | 23.773 | 24.158 | 23.773 | (0.40) |
| 8/23/2012 22:00 | 23.388 | 23.388 | 23.581 | 23.581 | | 9/5/2012 22:00 | 22.812 | 22.812 | 22.908 | 23.1 | |
| 8/24/2012 4:00 | 23.388 | 23.484 | 23.581 | 23.581 | | 9/6/2012 4:00 | 22.142 | 22.238 | 22.238 | 22.812 | |
| 8/24/2012 10:00 | 24.255 | 23.869 | 23.869 | 23.484 | 24.18 | 9/6/2012 10:00 | 22.812 | 22.717 | 22.621 | 22.429 | 22.65 |
| 8/24/2012 16:00 | 24.835 | 24.738 | 25.708 | 25.125 | (0.64) | 9/6/2012 16:00 | 22.908 | 22.812 | 22.908 | 23.388 | (0.30) |
| 8/24/2012 22:00 | 24.062 | 24.158 | 24.255 | 24.448 | | 9/6/2012 22:00 | 22.525 | 22.525 | 22.621 | 22.717 | |
| 8/25/2012 4:00 | 23.581 | 23.581 | 23.677 | 23.869 | | 9/7/2012 4:00 | 21.951 | 22.046 | 22.046 | 22.429 | |
| 8/25/2012 10:00 | 23.292 | 23.388 | 23.388 | 23.484 | 23.76 | 9/7/2012 10:00 | 22.812 | 22.621 | 22.525 | 21.855 | 22.16 |
| 8/25/2012 16:00 | 24.062 | 23.966 | 24.158 | 23.773 | (0.27) | 9/7/2012 16:00 | 22.333 | 22.238 | 22.333 | 22.621 | (0.37) |
| 8/25/2012 22:00 | 23.869 | 23.966 | 23.966 | 24.158 | | 9/7/2012 22:00 | 21.664 | 21.76 | 21.76 | 21.569 | |
| 8/26/2012 4:00 | 23.484 | 23.581 | 23.677 | 23.388 | | 9/8/2012 4:00 | 20.805 | 20.901 | 20.901 | 21.091 | |
| 8/26/2012 10:00 | 23.677 | 23.484 | 23.581 | 23.292 | 23.93 | 9/8/2012 10:00 | 20.996 | 20.901 | 20.996 | 20.805 | 21.12 |
| 8/26/2012 16:00 | 24.641 | 24.738 | 24.931 | 24.835 | (0.53) | 9/8/2012 16:00 | 21.664 | 21.76 | 21.855 | 22.238 | (0.48) |
| 8/26/2012 22:00 | 23.773 | 23.869 | 23.966 | 23.966 | | 9/8/2012 22:00 | 20.519 | 20.71 | 20.615 | 21.187 | |
| 8/27/2012 4:00 | 23.677 | 23.677 | 23.773 | 23.869 | | 9/9/2012 4:00 | 20.329 | 20.424 | 20.519 | 20.519 | |
| 8/27/2012 10:00 | 24.641 | 24.158 | 24.158 | 23.773 | 24.39 | 9/9/2012 10:00 | 20.901 | 20.805 | 20.71 | 20.424 | 20.97 |
| 8/27/2012 16:00 | 25.222 | 25.028 | 25.513 | 25.222 | (0.58) | 9/9/2012 16:00 | 21.091 | 21.473 | 21.76 | 21.855 | (0.46) |
| 8/27/2012 22:00 | 24.351 | 24.158 | 24.545 | 24.448 | | 9/9/2012 22:00 | 21.091 | 21.091 | 21.187 | 21.282 | |
| 8/28/2012 4:00 | 23.869 | 23.966 | 24.062 | 24.158 | | 9/10/2012 4:00 | 20.615 | 20.71 | 20.805 | 20.71 | |
| 8/28/2012 10:00 | 24.351 | 23.869 | 23.966 | 23.773 | 24.19 | 9/10/2012 10:00 | 20.901 | 20.901 | 20.901 | 20.71 | 20.84 |
| 8/28/2012 16:00 | 24.545 | 24.641 | 24.738 | 24.545 | (0.29) | 9/10/2012 16:00 | 20.901 | 21.187 | 21.187 | 21.282 | (0.22) |
| 8/28/2012 22:00 | 24.062 | 24.062 | 24.158 | 24.255 | | 9/10/2012 22:00 | 20.519 | 20.615 | 20.71 | 20.71 | |
| 8/29/2012 4:00 | 23.484 | 23.581 | 23.677 | 23.869 | | 9/11/2012 4:00 | 20.234 | 20.329 | 20.424 | 20.519 | |
| 8/29/2012 10:00 | 24.158 | 23.773 | 23.773 | 23.484 | 24.07 | 9/11/2012 10:00 | 20.519 | 20.519 | 20.615 | 20.424 | 20.63 |
| 8/29/2012 16:00 | 24.641 | 24.835 | 25.125 | 24.641 | (0.48) | 9/11/2012 16:00 | 21.091 | 21.282 | 21.187 | 21.282 | (0.35) |
| 8/29/2012 22:00 | 23.869 | 23.966 | 24.062 | 24.158 | | 9/11/2012 22:00 | 20.329 | 20.329 | 20.424 | 20.519 | |

Item Number 1
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File Folder: 17a

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 24 & 28) | Created...... | 4-Feb-14 | TJS TJS |
| Data Source: File Folder: 17a | Revised...... | 19-Feb-14 | TJS TJS |
| Forms: Onset HOBO Datafile output from HOBO Temperature Loggers (File Folder 18) | Reviewed... | 19FEB14 | TJS |
| (I:\AEH-12-PSEUDO-04\Data\Hobos\Lake Shawano HOBO Files) | Certified... | 2/19/14 | J |
| File Name: I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Lake Temperature Data Loggers.xlsx]Temp Data | | | |

**Temperature Data Loggers (HOBOs)**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6 and 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank and Bottom Injection

Data Explanation:
Upon exposure termination on September 6, 2012 (whole tank) and September 8, 2012 (bottom injection) at Lake Shawano, four wire mesh cages containing test animals were placed into Lake Shawano for a post-exposure holding period. Each cage was fitted with a HOBO temperature logger programmed to record water temperature every six hours (four times per day). Mean daily temperature (°C) and standard deviation were calculated from all daily

| Data Logger Number | Serial Number | Lake | Treatment Type | Treatment Duration |
|---|---|---|---|---|
| 1 | 10020137 | Shawano | Whole Tank | 6h |
| 2 | 10020138 | Shawano | Whole Tank | 9h |
| 3 | 10020134 | Shawano | Whole Tank | 12h |
| 4 | 10020136 | Shawano | Bottom Injection | 12h |

| Date | Mean Temperature (°C) | (STD) |
|---|---|---|
| 9/8/2012 | 21.49 | 0.65 |
| 9/9/2012 | 20.16 | 0.39 |
| 9/10/2012 | 20.95 | 0.78 |
| 9/11/2012 | 20.93 | 0.61 |
| 9/12/2012 | 20.69 | 0.24 |
| 9/13/2012 | 19.81 | 0.39 |
| 9/14/2012 | 19.44 | 0.43 |
| 9/15/2012 | 19.29 | 0.68 |
| 9/16/2012 | 19.91 | 0.30 |
| 9/17/2012 | 18.96 | 0.49 |
| 9/18/2012 | 17.17 | 0.43 |
| 9/19/2012 | 16.20 | 0.09 |
| 9/20/2012 | 15.47 | 0.29 |
| 9/21/2012 | 15.17 | 0.19 |
| 9/22/2012 | 14.39 | 0.43 |
| 9/23/2012 | 13.94 | 0.31 |
| 9/24/2012 | 13.41 | 0.15 |
| 9/25/2012 | 13.54 | 0.52 |
| 9/26/2012 | 14.43 | 0.36 |
| 9/27/2012 | 14.33 | 0.37 |
| 9/28/2012 | 15.08 | 0.25 |
| 9/29/2012 | 15.50 | 0.34 |
| 9/30/2012 | 15.02 | 0.41 |
| 10/1/2012 | 15.58 | 0.39 |
| 10/2/2012 | 15.81 | 0.33 |
| 10/3/2012 | 16.08 | 0.30 |
| 10/4/2012 | 16.33 | 0.24 |
| 10/5/2012 | 13.58 | 0.67 |
| 10/6/2012 | 11.20 | 0.60 |
| 10/7/2012 | 11.04 | 0.66 |
| 10/8/2012 | 10.52 | 0.53 |
| 10/9/2012 | 10.07 | 0.27 |

Data anomalies and deviations:
Temperature readings from the first and last days of recorder deployment (prior to September 8 and after October 9, 2012 for whole tank; prior to September 10 and after October 9, 2012 for bottom injection) were omitted from data analysis. The temperature recorders may have sampled ambient air temperature during these time points. These entire days will be omitted as diurnal temperature flucuation skew the mean daily temperature. The mean temperature for September 8 and 9, 2012 was calculated using only the three data loggers (#1, 2, and 3) from whole tank treatments.

Item Number 2
Page 1 of 2

**File Folder:** 17a

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 24 & 28)
Data Source: File Folder: 17a
Forms: HOBO Output in Excel "Lake Shawano 6h whole tank", "Lake Shawano 9h whole tank' "Lake Shawano 12h whole tank", "Lake Shawano 12h bottom injection"
Location: I:\AEH-12-PSEUDO-04\Data\Hobos\Carlos and Shawano in Excel

Test Article: MBI 401 SDP (*Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6 and 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank and Bottom Injection

## Post-Exposure Holding Period Water Temperature

| Date | Data Logger Number | | | | Mean | Date | Data Logger Number | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (GMT-05:00) | 1 | 2 | 3 | 4 | (STD) | Time (GMT-05:00) | 1 | 2 | 3 | 4 | (STD) |
| | Temperature (°C) | | | | | | Temperature (°C) | | | | |
| 9/8/2012 3:23 | 21.187 | 21.187 | 21.091 | . | | 9/24/2012 3:23 | 13.461 | 13.461 | 13.365 | 13.654 | |
| 9/8/2012 9:23 | 20.71 | 20.71 | 20.615 | . | 21.49 | 9/24/2012 9:23 | 13.173 | 13.173 | 13.076 | 13.365 | 13.41 |
| 9/8/2012 15:23 | 22.429 | 22.429 | 22.429 | . | (0.65) | 9/24/2012 15:23 | 13.461 | 13.461 | 13.365 | 13.365 | (0.15) |
| 9/8/2012 21:23 | 21.76 | 21.664 | 21.664 | . | | 9/24/2012 21:23 | 13.558 | 13.558 | 13.461 | 13.558 | |
| 9/9/2012 3:23 | 20.519 | 20.519 | 20.519 | . | | 9/25/2012 3:23 | 13.173 | 13.173 | 13.173 | 13.461 | |
| 9/9/2012 9:23 | 19.567 | 19.472 | 19.472 | . | 20.16 | 9/25/2012 9:23 | 12.883 | 12.883 | 12.787 | 12.883 | 13.54 |
| 9/9/2012 15:23 | 20.329 | 20.329 | 20.234 | . | (0.39) | 9/25/2012 15:23 | 14.23 | 14.134 | 14.038 | 13.75 | (0.52) |
| 9/9/2012 21:23 | 20.329 | 20.424 | 20.234 | . | | 9/25/2012 21:23 | 14.038 | 14.134 | 13.942 | 13.942 | |
| 9/10/2012 3:23 | 20.424 | 20.424 | 20.329 | 20.234 | | 9/26/2012 3:23 | 14.421 | 14.517 | 14.421 | 13.846 | |
| 9/10/2012 9:23 | 20.138 | 20.138 | 20.043 | 20.043 | 20.95 | 9/26/2012 9:23 | 14.038 | 14.134 | 13.942 | 13.942 | 14.43 |
| 9/10/2012 15:23 | 21.951 | 21.951 | 21.855 | 20.805 | (0.78) | 9/26/2012 15:23 | 14.996 | 14.804 | 14.9 | 14.23 | (0.36) |
| 9/10/2012 21:23 | 21.664 | 21.569 | 21.569 | 22.046 | | 9/26/2012 21:23 | 14.709 | 14.709 | 14.613 | 14.709 | |
| 9/11/2012 3:23 | 20.805 | 20.71 | 20.71 | 21.187 | | 9/27/2012 3:23 | 14.038 | 14.038 | 13.942 | 14.325 | |
| 9/11/2012 9:23 | 20.043 | 20.043 | 19.948 | 20.329 | 20.93 | 9/27/2012 9:23 | 13.942 | 14.038 | 13.846 | 13.846 | 14.33 |
| 9/11/2012 15:23 | 21.569 | 21.473 | 21.473 | 20.424 | (0.61) | 9/27/2012 15:23 | 14.996 | 14.709 | 14.804 | 14.325 | (0.37) |
| 9/11/2012 21:23 | 21.473 | 21.473 | 21.473 | 21.76 | | 9/27/2012 21:23 | 14.613 | 14.804 | 14.517 | 14.517 | |
| 9/12/2012 3:23 | 20.805 | 20.805 | 20.71 | 21.187 | | 9/28/2012 3:23 | 14.996 | 14.996 | 14.9 | 15.187 | |
| 9/12/2012 9:23 | 20.329 | 20.329 | 20.234 | 20.519 | 20.69 | 9/28/2012 9:23 | 14.804 | 14.9 | 14.709 | 14.709 | 15.08 |
| 9/12/2012 15:23 | 20.805 | 20.901 | 20.71 | 20.519 | (0.24) | 9/28/2012 15:23 | 15.569 | 15.378 | 15.378 | 14.9 | (0.25) |
| 9/12/2012 21:23 | 20.805 | 20.901 | 20.805 | 20.615 | | 9/28/2012 21:23 | 15.187 | 15.282 | 15.187 | 15.187 | |
| 9/13/2012 3:23 | 20.329 | 20.424 | 20.329 | 20.519 | | 9/29/2012 3:23 | 15.378 | 15.378 | 15.282 | 15.187 | |
| 9/13/2012 9:23 | 19.472 | 19.472 | 19.377 | 20.043 | 19.81 | 9/29/2012 9:23 | 15.282 | 15.378 | 15.187 | 15.187 | 15.50 |
| 9/13/2012 15:23 | 19.758 | 19.758 | 19.662 | 19.282 | (0.39) | 9/29/2012 15:23 | 16.141 | 15.951 | 16.046 | 15.569 | (0.34) |
| 9/13/2012 21:23 | 19.567 | 19.567 | 19.567 | 19.758 | | 9/29/2012 21:23 | 15.378 | 15.378 | 15.187 | 16.141 | |
| 9/14/2012 3:23 | 19.187 | 19.187 | 19.092 | 19.377 | | 9/30/2012 3:23 | 14.9 | 14.9 | 14.804 | 14.996 | |
| 9/14/2012 9:23 | 18.996 | 18.996 | 18.901 | 18.901 | 19.44 | 9/30/2012 9:23 | 14.517 | 14.613 | 14.421 | 14.517 | 15.02 |
| 9/14/2012 15:23 | 19.948 | 20.234 | 19.853 | 19.187 | (0.43) | 9/30/2012 15:23 | 15.378 | 15.187 | 15.282 | 14.804 | (0.41) |
| 9/14/2012 21:23 | 19.758 | 20.043 | 19.758 | 19.567 | | 9/30/2012 21:23 | 15.569 | 15.855 | 15.569 | 14.996 | |
| 9/15/2012 3:23 | 19.187 | 19.187 | 19.092 | 19.472 | | 10/1/2012 3:23 | 15.187 | 15.282 | 15.187 | 15.473 | |
| 9/15/2012 9:23 | 18.331 | 18.331 | 18.236 | 18.521 | 19.29 | 10/1/2012 9:23 | 15.282 | 15.282 | 15.187 | 14.996 | 15.58 |
| 9/15/2012 15:23 | 19.662 | 20.138 | 19.567 | 18.711 | (0.68) | 10/1/2012 15:23 | 16.141 | 16.046 | 15.951 | 15.473 | (0.39) |
| 9/15/2012 21:23 | 19.853 | 20.519 | 19.948 | 19.853 | | 10/1/2012 21:23 | 16.046 | 16.141 | 15.951 | 15.664 | |
| 9/16/2012 3:23 | 19.948 | 19.853 | 19.853 | 20.138 | | 10/2/2012 3:23 | 15.664 | 15.664 | 15.569 | 15.664 | |
| 9/16/2012 9:23 | 19.472 | 19.472 | 19.377 | 19.567 | 19.91 | 10/2/2012 9:23 | 15.473 | 15.473 | 15.378 | 15.473 | 15.81 |
| 9/16/2012 15:23 | 20.329 | 20.329 | 20.234 | 19.758 | (0.30) | 10/2/2012 15:23 | 16.237 | 16.046 | 16.046 | 15.569 | (0.33) |
| 9/16/2012 21:23 | 20.043 | 20.043 | 19.948 | 20.138 | | 10/2/2012 21:23 | 16.332 | 16.332 | 16.237 | 15.855 | |
| 9/17/2012 3:23 | 19.472 | 19.472 | 19.472 | 19.758 | | 10/3/2012 3:23 | 15.951 | 15.855 | 15.855 | 16.046 | |
| 9/17/2012 9:23 | 19.092 | 19.092 | 18.996 | 19.187 | 18.96 | 10/3/2012 9:23 | 15.76 | 15.76 | 15.664 | 15.76 | 16.08 |
| 9/17/2012 15:23 | 18.996 | 18.996 | 18.901 | 18.996 | (0.49) | 10/3/2012 15:23 | 16.618 | 16.332 | 16.427 | 15.855 | (0.30) |
| 9/17/2012 21:23 | 18.14 | 18.14 | 18.045 | 18.616 | | 10/3/2012 21:23 | 16.332 | 16.523 | 16.332 | 16.237 | |
| 9/18/2012 3:23 | 17.284 | 17.284 | 17.189 | 17.855 | | 10/4/2012 3:23 | 16.237 | 16.332 | 16.237 | 16.237 | |
| 9/18/2012 9:23 | 16.808 | 16.808 | 16.713 | 16.903 | 17.17 | 10/4/2012 9:23 | 16.237 | 16.237 | 16.141 | 16.141 | 16.33 |
| 9/18/2012 15:23 | 17.76 | 17.76 | 17.665 | 16.713 | (0.43) | 10/4/2012 15:23 | 16.713 | 16.713 | 16.618 | 16.523 | (0.24) |
| 9/18/2012 21:23 | 16.808 | 16.713 | 16.713 | 17.665 | | 10/4/2012 21:23 | 16.046 | 16.046 | 16.046 | 16.713 | |
| 9/19/2012 3:23 | 16.141 | 16.141 | 16.141 | 16.141 | | 10/5/2012 3:23 | 14.421 | 14.421 | 14.325 | 15.282 | |
| 9/19/2012 9:23 | 16.141 | 16.141 | 16.046 | 16.141 | 16.20 | 10/5/2012 9:23 | 13.461 | 13.461 | 13.365 | 13.461 | 13.58 |
| 9/19/2012 15:23 | 16.332 | 16.332 | 16.237 | 16.141 | (0.09) | 10/5/2012 15:23 | 13.461 | 13.461 | 13.365 | 12.98 | (0.67) |
| 9/19/2012 21:23 | 16.237 | 16.237 | 16.237 | 16.427 | | 10/5/2012 21:23 | 12.883 | 12.883 | 12.787 | 13.269 | |
| 9/20/2012 3:23 | 15.855 | 15.855 | 15.76 | 16.046 | | 10/6/2012 3:23 | 11.334 | 11.431 | 11.236 | 12.401 | |
| 9/20/2012 9:23 | 15.282 | 15.282 | 15.187 | 15.76 | 15.47 | 10/6/2012 9:23 | 10.748 | 10.748 | 10.651 | 10.553 | 11.20 |
| 9/20/2012 15:23 | 15.569 | 15.473 | 15.473 | 15.187 | (0.29) | 10/6/2012 15:23 | 12.013 | 12.013 | 11.916 | 11.236 | (0.60) |
| 9/20/2012 21:23 | 15.187 | 15.187 | 15.091 | 15.282 | | 10/6/2012 21:23 | 10.553 | 10.553 | 10.553 | 11.334 | |
| 9/21/2012 3:23 | 15.091 | 15.091 | 15.091 | 15.091 | | 10/7/2012 3:23 | 10.455 | 10.455 | 10.357 | 10.748 | |
| 9/21/2012 9:23 | 15.187 | 15.187 | 15.091 | 14.996 | 15.17 | 10/7/2012 9:23 | 10.553 | 10.553 | 10.455 | 10.357 | 11.04 |
| 9/21/2012 15:23 | 15.569 | 15.473 | 15.473 | 15.378 | (0.19) | 10/7/2012 15:23 | 12.207 | 12.207 | 12.11 | 11.139 | (0.66) |
| 9/21/2012 21:23 | 14.996 | 14.996 | 14.9 | 15.187 | | 10/7/2012 21:23 | 11.139 | 11.139 | 11.041 | 11.722 | |
| 9/22/2012 3:23 | 14.325 | 14.325 | 14.325 | 14.804 | | 10/8/2012 3:23 | 10.259 | 10.357 | 10.259 | 10.748 | |
| 9/22/2012 9:23 | 13.75 | 13.75 | 13.654 | 14.038 | 14.39 | 10/8/2012 9:23 | 9.866 | 9.866 | 9.768 | 9.768 | 10.52 |
| 9/22/2012 15:23 | 14.9 | 14.804 | 14.804 | 13.942 | (0.43) | 10/8/2012 15:23 | 11.334 | 11.334 | 11.236 | 10.553 | (0.53) |
| 9/22/2012 21:23 | 14.613 | 14.613 | 14.517 | 14.996 | | 10/8/2012 21:23 | 10.651 | 10.651 | 10.553 | 11.139 | |
| 9/23/2012 3:23 | 14.23 | 14.134 | 14.134 | 14.23 | | 10/9/2012 3:23 | 10.357 | 10.357 | 10.259 | 10.651 | |
| 9/23/2012 9:23 | 13.461 | 13.461 | 13.365 | 13.846 | 13.94 | 10/9/2012 9:23 | 10.063 | 10.063 | 9.965 | 10.259 | 10.07 |
| 9/23/2012 15:23 | 14.421 | 14.23 | 14.23 | 13.75 | (0.31) | 10/9/2012 15:23 | 10.161 | 10.161 | 10.063 | 10.063 | (0.27) |
| 9/23/2012 21:23 | 13.846 | 13.846 | 13.75 | 14.134 | | 10/9/2012 21:23 | 9.768 | 9.768 | 9.669 | 9.571 | |

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**File Folder:** 17a

# Appendix 7. Spectrophotometric Summary, SAS Program, Output and Log

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 10 - 12) | Created...... | 6-Feb-14 | TJS |
| Data Source: File Folder: 9c | Revised...... | 23-Apr-14 | KLW |
| Forms: "Sample Absorbance Readings" Data Sheet | Reviewed... | 23APR2014 | |
| | Certified...... | | |
| File Name: See filenames as stated below | | | |

Spectrophotometric Data

Test Article: Zequanox ® (MBI-401 SDP)
Test Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

**Data Explanation:**

1) The absorbance of triplicate samples of 25, 50, 100, and 200 mg/L dilutions of a 2,000 mg/L active ingredient (A.I.) stock prepared from Analytical Stock #1 were measured to prepare a standard curve.
2) Standard checks were performed at 9 and 12 hours by comparing the 25, 50, 100, and 200 mg/L (A.I.) dilutions to the linear curve.
3) Data codes used within SAS
   - tank = Tank ID (1 through 9)
   - thero = Theoretical or target concentration (mg/L)
   - time = Sample Time (0, 1, 3, 6, 9, and 12 h after treatment)
   - loc = Sample Location
     - sus = Suspended Sample (sampled ~15 cm from bottom of tank)
     - sur = Surface Sample
   - abs = measured absorbance of sample
   - conc = concentration (mg/L), only used for standards used for regression)

4) Information that is not relevant to a sample (i.e., tank ID for standards) or that will be calculated by SAS (i.e., predicted concentration for standard checks and samples) is denoted by a "." in the SAS input and output files.

**Data Analysis:**

1) A linear regression was completed in SAS using the absorbance values obtained from the spectrophotometer of 3 replicate dilutions of 25, 50, 100 and 200 mg/L Zequanox
2) Standard checks and treatment sample concentrations were predicted in SAS by comparing the observed absorbances with the linear regression.
3) The following mean treatment concentrations were determined in SAS:
   - 3a) Mean (standard deviation) concentration by tank for all sampling times
   - 3b) Mean (standard deviation) concentration by treatment group for all sampling times
   - 3c) Mean (standard deviation) concentration by treatment group and sampling times
   - 3d) Mean (standard deviation) concentrations for 25, 50, 100, and 200 mg/L (A.I.) dilutions for all sampling times

**File Names:**

Spectrophotometric Data for SAS Input
I:\AEH-12-PSEUDO-04\Data Summaries\spec\[Lake Carlos Whole Tank Spec Summary.xlsx]Spec Data for SAS

SAS Program/Code
I:\AEH-12-PSEUDO-04\SAS-Spec\carlos whole water program file

SAS Log
I:\AEH-12-PSEUDO-04\SAS-Spec\carlos whole water log file

SAS Output
I:\AEH-12-PSEUDO-04\SAS-Spec\carlos whole water results file

**Data Anomalies and Deviations:**

1) One exposure tank of a different concentration and sample location was sampled in triplicate to evaluate variability of spectrophotometer during each sampling time. The mean absorbance of the triplicate samples was imported into SAS for use in the analysis.
2) Samples were collected by submerging a collection beaker below the surface the surface of each exposure tank. Care was taken to avoid foam or particles from surface of water.
3) Some mean absorbances for triplicate samples may be recorded incorrectly on "Sample Absorbance Readings" data forms as proper significant figure rules may not have been observed. Additionally, concentrations recorded on "Sample Absorbance Readings" data forms were not used in the analysis as the initial linear regression equation that was used for these calculations was derived using rounded absorbance values in Excel. All absorbances and concentrations used in SAS calculations and reported within Spectrophotometric Data Summary have been corrected.

Item Number 1
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File Folder: 9c

AEH-12-PSEUDO-04

| tank | thero | time | loc | abs | conc |
|---|---|---|---|---|---|
| . | 25 | 0 | . | 0.038 | 25 |
| . | 50 | 0 | . | 0.077 | 50 |
| . | 100 | 0 | . | 0.152 | 100 |
| . | 200 | 0 | . | 0.291 | 200 |
| . | 25 | 0 | . | 0.040 | 25 |
| . | 50 | 0 | . | 0.077 | 50 |
| . | 100 | 0 | . | 0.149 | 100 |
| . | 200 | 0 | . | 0.291 | 200 |
| . | 25 | 0 | . | 0.040 | 25 |
| . | 50 | 0 | . | 0.076 | 50 |
| . | 100 | 0 | . | 0.148 | 100 |
| . | 200 | 0 | . | 0.290 | 200 |
| . | 25 | 9 | . | 0.035 | . |
| . | 50 | 9 | . | 0.069 | . |
| . | 100 | 9 | . | 0.139 | . |
| . | 200 | 9 | . | 0.269 | . |
| . | 25 | 12 | . | 0.034 | . |
| . | 50 | 12 | . | 0.074 | . |
| . | 100 | 12 | . | 0.136 | . |
| . | 200 | 12 | . | 0.266 | . |
| 1 | 50 | 1 | sur | 0.077 | . |
| 4 | 50 | 1 | sur | 0.078 | . |
| 8 | 50 | 1 | sur | 0.084 | . |
| 6 | 100 | 1 | sur | 0.160 | . |
| 7 | 100 | 1 | sur | 0.155 | . |
| 9 | 100 | 1 | sur | 0.154 | . |
| 1 | 50 | 3 | sus | 0.071 | . |
| 4 | 50 | 3 | sus | 0.074 | . |
| 8 | 50 | 3 | sus | 0.078 | . |
| 6 | 100 | 3 | sus | 0.142 | . |
| 7 | 100 | 3 | sus | 0.138 | . |
| 9 | 100 | 3 | sus | 0.139 | . |
| 1 | 50 | 3 | sur | 0.070 | . |
| 4 | 50 | 3 | sur | 0.073 | . |
| 8 | 50 | 3 | sur | 0.078 | . |
| 6 | 100 | 3 | sur | 0.142 | . |
| 7 | 100 | 3 | sur | 0.137 | . |
| 9 | 100 | 3 | sur | 0.140 | . |
| 1 | 50 | 6 | sus | 0.065 | . |
| 4 | 50 | 6 | sus | 0.063 | . |
| 8 | 50 | 6 | sus | 0.065 | . |
| 6 | 100 | 6 | sus | 0.132 | . |
| 7 | 100 | 6 | sus | 0.131 | . |
| 9 | 100 | 6 | sus | 0.133 | . |
| 1 | 50 | 6 | sur | 0.063 | . |
| 4 | 50 | 6 | sur | 0.062 | . |

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| | | | | | |
|---|---|---|---|---|---|
| 8 | 50 | 6 | sur | 0.067 | . |
| 6 | 100 | 6 | sur | 0.127 | . |
| 7 | 100 | 6 | sur | 0.128 | . |
| 9 | 100 | 6 | sur | 0.129 | . |
| 1 | 50 | 9 | sus | 0.063 | . |
| 4 | 50 | 9 | sus | 0.061 | . |
| 8 | 50 | 9 | sus | 0.065 | . |
| 6 | 100 | 9 | sus | 0.126 | . |
| 7 | 100 | 9 | sus | 0.126 | . |
| 9 | 100 | 9 | sus | 0.126 | . |
| 1 | 50 | 9 | sur | 0.059 | . |
| 4 | 50 | 9 | sur | 0.059 | . |
| 8 | 50 | 9 | sur | 0.063 | . |
| 6 | 100 | 9 | sur | 0.126 | . |
| 7 | 100 | 9 | sur | 0.122 | . |
| 9 | 100 | 9 | sur | 0.124 | . |
| 1 | 50 | 12 | sus | 0.058 | . |
| 4 | 50 | 12 | sus | 0.057 | . |
| 8 | 50 | 12 | sus | 0.062 | . |
| 6 | 100 | 12 | sus | 0.124 | . |
| 7 | 100 | 12 | sus | 0.121 | . |
| 9 | 100 | 12 | sus | 0.118 | . |
| 1 | 50 | 12 | sur | 0.060 | . |
| 4 | 50 | 12 | sur | 0.058 | . |
| 8 | 50 | 12 | sur | 0.062 | . |
| 6 | 100 | 12 | sur | 0.125 | . |
| 7 | 100 | 12 | sur | 0.120 | . |
| 9 | 100 | 12 | sur | 0.120 | . |

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Item No. 1
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```
ods html close; /* close previous */;
ods html; /* open new */;
ods graphics on;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Lake Carlos-whole water treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  23APR2014  Analysis prepare

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  KLW klw   Page 1 of ___
* Analysis completion date:  23 April 2014  23APR2014
*****************************************************************************

data Zeq; set carlos.carloswhole;
run;
proc sort;
by tank time loc; run;

run;
proc gplot data= zeq;
plot abs * conc;
run;
proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
proc sort;
by time tank loc;
proc print data=output_out;
run;
data zeq2; set output_out;
if tank = "." then delete;
if time = "0" then delete;
if loc =".' then delete;
run;
proc sort;
by tank loc;
run;
/*****************************************************************************
 * This procedure produces the mean concentrations for each treatment replicat
```

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Item No. 2
Pg 1 of 3

```
 * by the the sampling location
 * i.e. It gives the mean concentration of each treatment tank over the entire
 * location [i.e. surface vs suspended sampling
 ****************************************************************************
title "Mean treatment concentration by treatment tank and sampling location (s
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;
proc sort;
by thero time loc;
/****************************************************************************
 * This procedure produces the mean concentrations for each treatment group ov
 * by the sampling location
 * i.e. It gives the mean concentration of the 3 50ppm & 100ppm treatment tank
 * sampling location (surface/suspended) over the entire exposure
 ****************************************************************************
title "Mean treatment concentration by treatment group and sampling location f
proc sort;
by thero loc time;

proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

data time9; set zeq2;
if time > 9 then delete;
run;
proc sort;
by thero loc time;
/****************************************************************************
 * This procedure produces the mean concentrations for each treatment group th
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks through th
 ****************************************************************************
title "Mean treatment concentration by treatment group and sampling location t
proc means data = time9 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;


data time6; set zeq2;
if time > 6 then delete;
run;
proc sort;
```

Page 2 of 3

```
by thero loc time;
/*******************************************************************************
 * This procedure produces the mean concentrations for each treatment group th
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks through th
 *******************************************************************************
title "Mean treatment concentration by treatment group and sampling location t
proc means data = time6 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

/*******************************************************************************
 * This procedure produces the mean concentrations for each treatment group by
 * i.e. It gives the mean conc. of the 3 50ppm & 100ppm  treatment tanks at ti
 *******************************************************************************
title "Mean treatment concentration for each treatment group for each sampling
proc sort;
by thero loc time;

proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc time;
var predicted_ppm;
run;

data zeq3; set output_out;
if conc > 1 then delete;
if tank > 0.5 then delete;
if thero = "." then delete;
run;

proc sort;
by thero;
/*******************************************************************************
 * This procedure produces the mean concentrations for the standard checks for
 * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at 9 anc
 *******************************************************************************
title "Mean concentration for standard checks for all sampling times";
proc means data = zeq3 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
run;
quit;
```

FF # 9c
Item No. 2
Pg 3 of 3

```
DM  LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

 FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Lake Carlos-whole water treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  23APR2014  Analysis pr

/*************************************************************************
* SAS ver 9.3        Analysis prepared by:  KLW KLW 23APR2014  Page  1  of  8
* Analysis completion date:  23 April 2014
**************************************************************************

data Zeq; set carlos.carloswhole;
run;

NOTE: There were 74 observations read from the data set CARLOS.CARLOSWHOLE.
NOTE: The data set WORK.ZEQ has 74 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by tank time loc; run;

NOTE: There were 74 observations read from the data set WORK.ZEQ.
NOTE: The data set WORK.ZEQ has 74 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds



run;
proc gplot data= zeq;
plot abs * conc;
run;

NOTE: 62 observation(s) contained a MISSING value for the abs * conc request.
NOTE: 4 records written to C:\Users\klweber\gplot.png.
```

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```
NOTE: There were 74 observations read from the data set WORK.ZEQ.
NOTE: PROCEDURE GPLOT used (Total process time):
      real time           0.82 seconds
      cpu time            0.50 seconds


29   proc reg data = zeq;
30   model conc = abs /edf;
31   output out=output_out p=predicted_ppm;
32   run;


NOTE: The data set WORK.OUTPUT_OUT has 74 observations and 7 variables.
NOTE: PROCEDURE REG used (Total process time):
      real time           2.60 seconds
      cpu time            0.62 seconds


33   proc sort;
34   by time tank loc;

NOTE: There were 74 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.OUTPUT_OUT has 74 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


35   proc print data=output_out;
36   run;

NOTE: There were 74 observations read from the data set WORK.OUTPUT_OUT.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.12 seconds
      cpu time            0.06 seconds


37   data zeq2; set output_out;
38   if tank = "." then delete;
39   if time = "0" then delete;
40   if loc =" ." then delete;
41   run;

NOTE: Character values have been converted to numeric values at the places giv
      (Line):(Column).
```

```
      39:11
NOTE: There were 74 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ2 has 54 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


42   proc sort;
43   by tank loc;
44   run;

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 54 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


45   /************************************************************************
45 ! **************
46    * This procedure produces the mean concentrations for each treatment rep
46 ! sampling times  *
47    * by the the sampling location
47 !             *
48    * i.e. It gives the mean concentration of each treatment tank over the e
48 ! the sampling *
49    * location [i.e. surface vs suspended sampling
49 !             *
50    ************************************************************************
50 ! **************/
51   title "Mean treatment concentration by treatment tank and sampling locati
51 ! (surface/suspended) for all sampling times';
52   proc means data = zeq2 mean std lclm uclm fw=8;
53   by tank loc;
54   var predicted_ppm;
55   run;

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.12 seconds
      cpu time            0.04 seconds


56   proc sort;
57   by thero time loc;
```

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```
58    /*************************************************************************
58  ! **************
59     * This procedure produces the mean concentrations for each treatment grc
59  ! sampling times       *
60     * by the sampling location
60  !             *
61     * i.e. It gives the mean concentration of the 3 50ppm & 100ppm treatment
61  !             *
62     * sampling location (surface/suspended) over the entire exposure
62  !             *
63    *************************************************************************
63  ! **************/
64   title "Mean treatment concentration by treatment group and sampling locat
64  ! sampling times';

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 54 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


65   proc sort;
66   by thero loc time;
67

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 54 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


68   proc means data = zeq2 mean std lclm uclm fw=8;
69   by thero loc;
70   var predicted_ppm;
71   run;

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.09 seconds
      cpu time            0.01 seconds


72
73   data time9; set zeq2;
```

```
if time > 9 then delete;
run;

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.TIME9 has 42 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by thero loc time;
/************************************************************************
*********************
 * This procedure produces the mean concentrations for each treatment grc
sample location      *
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks throu
 by sample location *
************************************************************************
*********************/
title "Mean treatment concentration by treatment group and sampling locat
 exposure";

NOTE: There were 42 observations read from the data set WORK.TIME9.
NOTE: The data set WORK.TIME9 has 42 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc means data = time9 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

NOTE: There were 42 observations read from the data set WORK.TIME9.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.09 seconds
      cpu time            0.01 seconds


data time6; set zeq2;
if time > 6 then delete;
run;
```

```
NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.TIME6 has 30 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


92   proc sort;
93   by thero loc time;
94   /***********************************************************************
94 ! ********************
95    * This procedure produces the mean concentrations for each treatment grc
95 ! sample location       *
96    * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks throu
96 !  by sample location *
97    ***********************************************************************
97 ! ********************/
98   title "Mean treatment concentration by treatment group and sampling locat
98 !  exposure";

NOTE: There were 30 observations read from the data set WORK.TIME6.
NOTE: The data set WORK.TIME6 has 30 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


99   proc means data = time6 mean std lclm uclm fw=8;
100  by thero loc;
101  var predicted_ppm;
102  run;

NOTE: There were 30 observations read from the data set WORK.TIME6.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.23 seconds
      cpu time            0.03 seconds


103
104  /***********************************************************************
104! **************
105   * This procedure produces the mean concentrations for each treatment grc
105!              *
106   * i.e. It gives the mean conc. of the 3 50ppm & 100ppm  treatment tanks
106! and 12h       *
```

```
107   ***********************************************************************
107!  *************/
108   title "Mean treatment concentration for each treatment group for each sam
109   proc sort;
110   by thero loc time;
111

NOTE: Input data set is already sorted, no sorting done.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


112   proc means data = zeq2 mean std lclm uclm fw=8;
113   by thero loc time;
114   var predicted_ppm;
115   run;

NOTE: There were 54 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.17 seconds
      cpu time            0.06 seconds


116
117   data zeq3; set output_out;
118   if conc > 1 then delete;
119   if tank > 0.5 then delete;
120   if thero = "." then delete;
121   run;

NOTE: Character values have been converted to numeric values at the places giv
      (Line):(Column).
      119:4    120:12
NOTE: There were 74 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ3 has 8 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


122
123   proc sort;
124   by thero;
125   /**********************************************************************
125!  *************
```

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```
126   * This procedure produces the mean concentrations ror the standard check
126!  periods           *
127   * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at
127!          *
128   ************************************************************************
128!  **************/
129   title 'Mean corcentration for standard chocks for all sampling times";

NOTE: There were 8 observations read from the data set WORK.ZEQ3.
NOTE: The data set WORK.ZEQ3 has 8 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


130   proc means data = zeq3 mean std lclm uclm fw=8;
131   by thero;
132   var predicted_ppm;
133   run;

NOTE: There were 8 observations read from the data set WORK.ZEQ3.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.04 seconds


134   quit;
```

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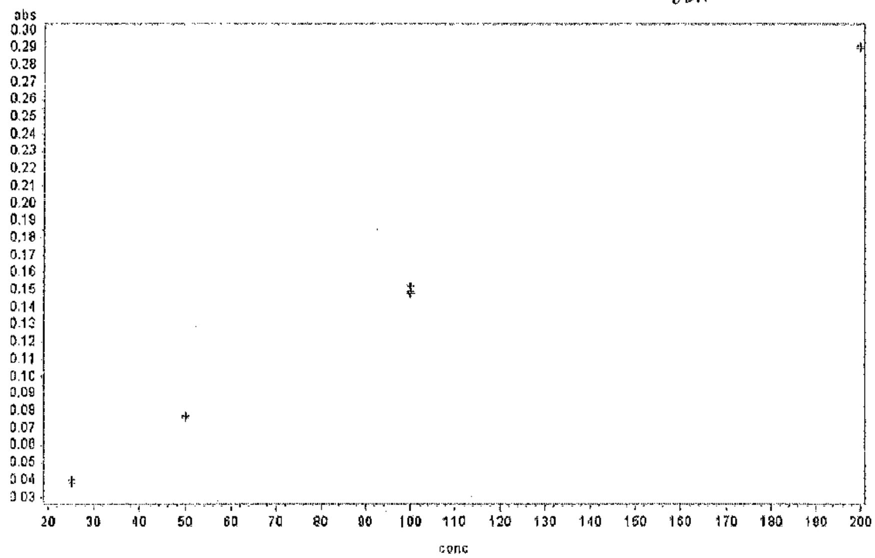
Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-whole water treatment
SAS v. 9.3 Analysis completion date: 23APR2014 Analysis prepared by: KLW KLW 23APR2014




Performed by K. Weber SAS version 9.3 09:49 23APR14

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Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-whole water treatment
SAS v. 9.3 Analysis completion date: 23APR2014 Analysis prepared by: KLW

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc

| | |
|---|---|
| Number of Observations Read | 74 |
| Number of Observations Used | 12 |
| Number of Observations with Missing Values | 62 |

Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 53893 | 53893 | 41456.9 | <.0001 |
| Error | 10 | 12.99981 | 1.29998 | | |
| Corrected Total | 11 | 53906 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 1.14017 | R-Square | 0.9998 |
| Dependent Mean | 93.75000 | Adj R-Sq | 0.9997 |
| Coeff Var | 1.21618 | | |

Parameter Estimates

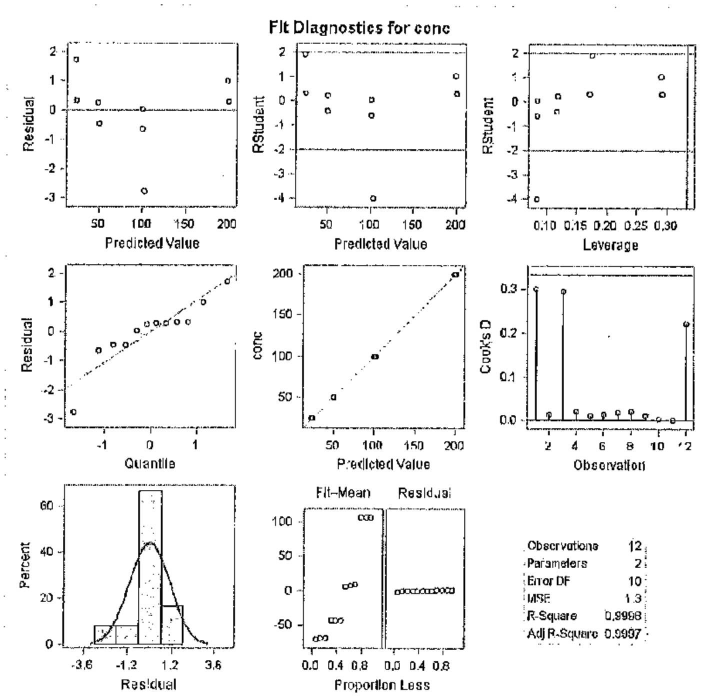
| Variable | Label | DF | Parameter Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| Intercept | Intercept | 1 | -3.24187 | 0.57901 | -5.60 | 0.0002 |
| abs | abs | 1 | 697.36515 | 3.42501 | 203.61 | <.0001 |

Performed by K. Weber SAS version 9.3 09:49 23APR14

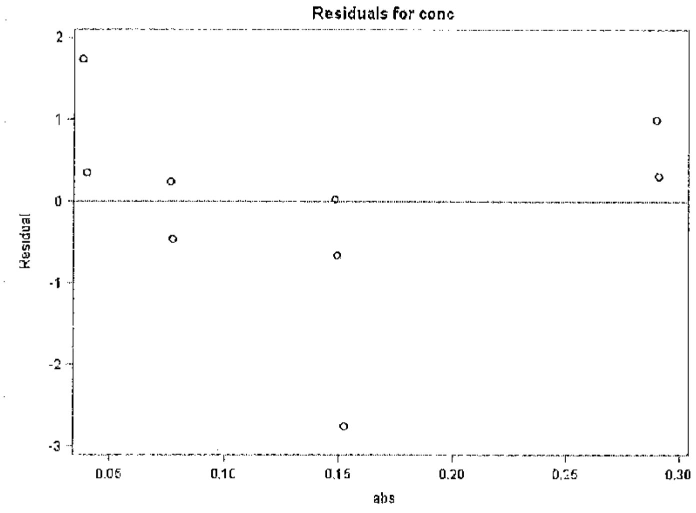
AEH-12-PSEUDO-04

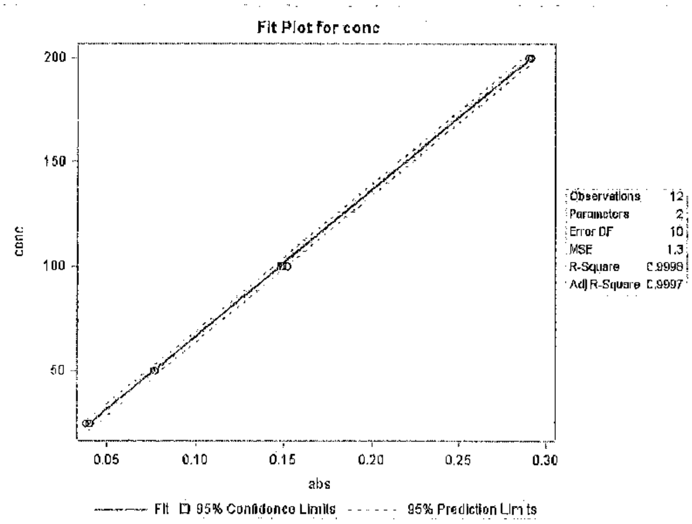
Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-whole water treatment
SAS v. 9.3 Analysis completion date: 23APR2014 Analysis prepared by: KLW

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc



AEH-12-PSEUDO-0;




Performed by K. Weber SAS version 9.3 09:49 23APR14

AEH-12-PSEUDO-0:

Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-whole water treatment
SAS v. 9.3 Analysis completion date: 23APR2014 Analysis prepared by: KLW

| Obs | tank | thero | time | loc | abs | conc | predicted_ppm |
|---|---|---|---|---|---|---|---|
| 1 | . | 25 | 0 | . | 0.038 | 25 | 23.258 |
| 2 | . | 50 | 0 | . | 0.077 | 50 | 50.455 |
| 3 | . | 100 | 0 | . | 0.152 | 100 | 102.758 |
| 4 | . | 200 | 0 | . | 0.291 | 200 | 199.691 |
| 5 | . | 25 | 0 | . | 0.040 | 25 | 24.653 |
| 6 | . | 50 | 0 | . | 0.077 | 50 | 50.455 |
| 7 | . | 100 | 0 | . | 0.149 | 100 | 100.666 |
| 8 | . | 200 | 0 | . | 0.291 | 200 | 199.691 |
| 9 | . | 25 | 0 | . | 0.040 | 25 | 24.653 |
| 10 | . | 50 | 0 | . | 0.076 | 50 | 49.758 |
| 11 | . | 100 | 0 | . | 0.148 | 100 | 99.968 |
| 12 | . | 200 | 0 | . | 0.290 | 200 | 198.994 |
| 13 | 1 | 50 | 1 | sur | 0.077 | . | 50.455 |
| 14 | 4 | 50 | 1 | sur | 0.078 | . | 51.153 |
| 15 | 6 | 100 | 1 | sur | 0.160 | . | 108.337 |
| 16 | 7 | 100 | 1 | sur | 0.155 | . | 104.850 |
| 17 | 8 | 50 | 1 | sur | 0.084 | . | 55.337 |
| 18 | 9 | 100 | 1 | sur | 0.154 | . | 104.152 |
| 19 | 1 | 50 | 3 | sur | 0.070 | . | 45.574 |
| 20 | 1 | 50 | 3 | sus | 0.071 | . | 46.271 |
| 21 | 4 | 50 | 3 | sur | 0.073 | . | 47.666 |
| 22 | 4 | 50 | 3 | sus | 0.074 | . | 48.363 |
| 23 | 6 | 100 | 3 | sur | 0.142 | . | 95.784 |
| 24 | 6 | 100 | 3 | sus | 0.142 | . | 95.784 |
| 25 | 7 | 100 | 3 | sur | 0.137 | . | 92.297 |
| 26 | 7 | 100 | 3 | sus | 0.138 | . | 92.995 |
| 27 | 8 | 50 | 3 | sur | 0.078 | . | 51.153 |
| 28 | 8 | 50 | 3 | sus | 0.078 | . | 51.153 |
| 29 | 9 | 100 | 3 | sur | 0.140 | . | 94.389 |
| 30 | 9 | 100 | 3 | sus | 0.139 | . | 93.692 |
| 31 | 1 | 50 | 6 | sur | 0.063 | . | 40.692 |
| 32 | 1 | 50 | 6 | sus | 0.065 | . | 42.087 |
| 33 | 4 | 50 | 6 | sur | 0.062 | . | 39.995 |
| 34 | 4 | 50 | 6 | sus | 0.063 | . | 40.692 |
| 35 | 6 | 100 | 6 | sur | 0.127 | . | 85.324 |
| 36 | 6 | 100 | 6 | sus | 0.132 | . | 88.810 |
| 37 | 7 | 100 | 6 | sur | 0.128 | . | 86.021 |
| 38 | 7 | 100 | 6 | sus | 0.131 | . | 88.113 |

AEH-12-PSEUDO-(:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 39 | 8 | 50 | 6 | sur | 0 067 | . | 43.482 |
| 40 | 8 | 50 | 6 | sus | 0.065 | . | 42.087 |
| 41 | 9 | 100 | 6 | sur | 0.129 | . | 86.718 |
| 42 | 9 | 100 | 6 | sus | 0.133 | . | 89.508 |
| 43 | . | 25 | 9 | . | 0.035 | . | 21.166 |
| 44 | . | 50 | 9 | . | 0.069 | . | 44.876 |
| 45 | . | 100 | 9 | . | 0.139 | . | 93.692 |
| 46 | . | 200 | 9 | . | 0.269 | . | 184.349 |
| 47 | 1 | 50 | 9 | sur | 0.059 | . | 37.903 |
| 48 | 1 | 50 | 9 | sus | 0.063 | . | 40.692 |
| 49 | 4 | 50 | 9 | sur | 0.059 | . | 37.903 |
| 50 | 4 | 50 | 9 | sus | 0.061 | . | 39.297 |
| 51 | 6 | 100 | 9 | sur | 0.126 | . | 84.626 |
| 52 | 6 | 100 | 9 | sus | 0.126 | . | 84.626 |
| 53 | 7 | 100 | 9 | sur | 0.122 | . | 81.837 |
| 54 | 7 | 100 | 9 | sus | 0.126 | . | 84.626 |
| 55 | 8 | 50 | 9 | sur | 0.063 | . | 40.692 |
| 56 | 8 | 50 | 9 | sus | 0.065 | . | 42.087 |
| 57 | 9 | 100 | 9 | sur | 0.124 | . | 83.231 |
| 58 | 9 | 100 | 9 | sus | 0.126 | . | 84.626 |
| 59 | . | 25 | 12 | . | 0.034 | . | 20.469 |
| 60 | . | 50 | 12 | . | 0.074 | . | 48.363 |
| 61 | . | 100 | 12 | . | 0.136 | . | 91.600 |
| 62 | . | 200 | 12 | . | 0.266 | . | 182.257 |
| 63 | 1 | 50 | 12 | sur | 0.060 | . | 38.600 |
| 64 | 1 | 50 | 12 | sus | 0.058 | . | 37.205 |
| 65 | 4 | 50 | 12 | sur | 0.058 | . | 37.205 |
| 66 | 4 | 50 | 12 | sus | 0.057 | . | 36.508 |
| 67 | 6 | 100 | 12 | sur | 0.125 | . | 83.929 |
| 68 | 6 | 100 | 12 | sus | 0.124 | . | 83.231 |
| 69 | 7 | 100 | 12 | sur | 0.120 | . | 80.442 |
| 70 | 7 | 100 | 12 | sus | 0.121 | . | 81.139 |
| 71 | 8 | 50 | 12 | sur | 0.062 | . | 39.995 |
| 72 | 8 | 50 | 12 | sus | 0.062 | . | 39.995 |
| 73 | 9 | 100 | 12 | sur | 0.120 | . | 80.442 |
| 74 | 9 | 100 | 12 | sus | 0.118 | . | 79.047 |

Performed by K. Weber SAS version 9.3 09:49 23APR14

AEH-12-PSEUDO-0[illegible]

**Mean treatment concentration by treatment tank and sampling location (surface/suspended) for all sampling times**

The MEANS Procedure

tank=1 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 42.6448 | 5.2972 | 36.0674 | 49.2221 |

tank=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 41.5638 | 3.7500 | 35.5967 | 47.5310 |

tank=4 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 42.7842 | 6.2569 | 35.0153 | 50.5532 |

tank=4 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 41.2152 | 5.0729 | 33.1430 | 49.2873 |

tank=6 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 91.5998 | 10.5415 | 78.5108 | 104.7 |

tank=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 88.1130 | 5.6367 | 79.1437 | 97.0823 |

tank=7 loc=sur

Analysis Variable : predicted_ppm Predicted

| Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 89.0893 | 9.9433 | 76.7431 | 101.4 |

tank=7 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 86.7182 | 5.0609 | 78.6652 | 94.7713 |

tank=8 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 46.1316 | 6.7863 | 37.7052 | 54.5579 |

tank=8 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.8303 | 4.9802 | 35.9057 | 51.7549 |

tank=9 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 89.7866 | 9.5821 | 77.8889 | 101.7 |

tank=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 86.7182 | 6.3149 | 76.6698 | 96.7667 |

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## Mean treatment concentration by treatment group and sampling location for all sampling times

**The MEANS Procedure**

**thero=50 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.8535 | 5.9283 | 40.5705 | 47.1365 |

**thero=50 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 42.2031 | 4.3686 | 39.4274 | 44.9787 |

**thero=100 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 90.1586 | 9.3504 | 84.9805 | 95.3367 |

**thero=100 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 87.1831 | 5.1960 | 83.8818 | 90.4845 |

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**Mean treatment concentration by treatment group and sampling location through the 9 h exposure**

The MEANS Procedure

thero=50 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 45.1669 | 5.9133 | 41.4098 | 48.9240 |

thero=50 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.6366 | 4.0178 | 40.5482 | 46.7250 |

thero=100 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 92.2972 | 9.2516 | 86.4190 | 98.1753 |

thero=100 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 89.1978 | 4.2148 | 85.9580 | 92.4375 |

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Mean treatment concentration by treatment group and sampling location through the 6 h exposure

The MEANS Procedure

thero=50 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 47.2784 | 5.2315 | 43.2571 | 51.2997 |

thero=50 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 45.1088 | 4.1531 | 40.7504 | 49.4672 |

thero=100 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 95.3191 | 8.7240 | 88.6132 | 102.0 |

thero=100 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 91.4836 | 3.1006 | 88.2298 | 94.7373 |

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Mean treatment concentration for each treatment group for each sampling time

The MEANS Procedure

thero=50 loc=sur time=1

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 52.3149 | 2.6402 | 45.7563 | 58.8735 |

thero=50 loc=sur time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 48.1307 | 2.8184 | 41.1295 | 55.1319 |

thero=50 loc=sur time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 41.3895 | 1.8451 | 36.8061 | 45.9729 |

thero=50 loc=sur time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 38.8325 | 1.6105 | 34.8318 | 42.8332 |

thero=50 loc=sur time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 38.6000 | 1.3947 | 35.1353 | 42.0647 |

thero=50 loc=sus time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 48.5956 | 2.4491 | 42.5118 | 54.6794 |

thero=50 loc=sus time=6

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 41.6220 | 0.8052 | 39.6216 | 43.6223 |

thero=50 loc=sus time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 40.6921 | 1.3947 | 37.2274 | 44.1568 |

thero=50 loc=sus time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 37.9027 | 1.8451 | 33.3193 | 42.4860 |

thero=100 loc=sur time=1

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 105.8 | 2.2417 | 100.2 | 111.3 |

thero=100 loc=sur time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 94.1568 | 1.7550 | 89.7971 | 98.5165 |

thero=100 loc=sur time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 86.0209 | 0.6974 | 84.2885 | 87.7532 |

thero=100 loc=sur time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 83.2314 | 1.3947 | 79.7667 | 86.6961 |

thero=100 loc=sur time=12

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 81.6042 | 2.0131 | 76.6034 | 86.6051 |

thero=100 loc=sus time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 94.1568 | 1.4517 | 90.5506 | 97.7630 |

thero=100 loc=sus time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 88.8103 | 0.6974 | 87.0780 | 90.5427 |

thero=100 loc=sus time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 84.6261 | 0 | . | . |

thero=100 loc=sus time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 81.1393 | 2.0921 | 75.9423 | 86.3364 |

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**Mean concentration for standard checks for all sampling times**

The MEANS Procedure

thero=25

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 20.8172 | 0.4931 | 16.3868 | 25.2477 |

thero=50

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 46.6197 | 2.4656 | 24.4676 | 68.7719 |

thero=100

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 92.6458 | 1.4793 | 79.3545 | 105.9 |

thero=200

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 183.3 | 1.4793 | 170.0 | 196.6 |

Performed by K. Weber SAS version 9.3 09:49 23APR14 KLW 23APR2014

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Item No. 4
Pg 15 of 15

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 17 - 23) | Created...... | 6-Feb-14 | T.S TS |
| Data Source: File Folder: 11c | Revised...... | 12-Feb-14 | T.S TS |
| Forms: "Sample Absorbance Readings" Data Sheet | Reviewed... | 12 FEB 14 | TS |
| | Certified...... | 2/19/14 | JS |
| File Name: See filenames as stated below | | | |

## Spectrophotometric Data

Test Article: Zequanox ® (MBI-401 SDP)
Test Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

**Data Explanation:**

1) The absorbance of triplicate samples of 25, 50, 100, and 200 mg/L dilutions of a 2,000 mg/L active ingredient (A.I.) stock prepared from Analytical Stock #1 were measured to prepare a standard curve.
2) Standard checks were performed at 6, 9, and 12 hours by comparing the 25, 50, 100, and 200 mg/L (A.I.) dilutions to the linear curve.
3) Data codes used within SAS
   - tank = Tank ID (1 through 9)
   - thero = Theoretical or target concentration (mg/L)
   - time = Sample Time (0, 1, 3, 6, 9, and 12 h after treatment)
   - loc = Sample Location
     - sus = Suspended Sample (sampled ~15 cm from bottom of tank)
     - sur = Surface Sample
   - abs = measured absorbance of sample
   - conc = concentration [(mg/L), only used for standards used for regression]

4) Information that is not relevant to a sample (i.e., tank ID for standards) or that will be calculated by SAS (i.e., predicted concentration for standard checks and samples) is denoted by a "." in the SAS input and output files.

**Data Analysis:**

1) A linear regression was completed in SAS using the absorbance values obtained from the spectrophotometer of 3 replicate dilutions of 25, 50, 100 and 200 mg/L Zequanox
2) Standard checks and treatment sample concentrations were predicted in SAS by comparing the observed absorbances with the linear regression.
3) The following mean treatment concentrations were determined in SAS:
   - 3a) Mean (standard deviation) concentration by tank and location for all sampling times
   - 3b) Mean (standard deviation) concentration by treatment group and location for all sampling times
   - 3c) Mean (standard deviation) concentration by treatment group and sampling times for both locations
   - 3d) Mean (standard deviation) concentrations for 25, 50, 100, and 200 mg/L (A.I.) dilutions for all sampling times

**File Names:**

Spectrophotometric Data for SAS Input
I:\AEH-12-PSEUDO-04\Data Summaries\spec\[Lake Shawano Whole Tank Spec Summary.xlsx]Spec Data for SAS

SAS Program/Code
I:\AEH-12-PSEUDO-04\SAS-Spec\shawano whole water program file

SAS Log
I:\AEH-12-PSEUDO-04\SAS-Spec\shawano whole water log file

SAS Output
I:\AEH-12-PSEUDO-04\SAS-Spec\shawano whole water results file

**Data Anomalies and Deviations:**

1) One exposure tank of a different concentration and sample location was sampled in triplicate to evaluate variability of spectrophotometer during each sampling time. The mean absorbance of the triplicate samples was imported into SAS for use in the analysis.
2) Surface samples (sur) were collected by submerging a collection beaker below the surface of each exposure tank; suspended samples (sus) were collected ~15 cm from the exposure tank bottom for all sampling times.
3) Some mean absorbances for triplicate samples may be recorded incorrectly on "Sample Absorbance Readings" data forms as proper significant figure rules may not have been observed. Additionally, concentrations recorded on "Sample Absorbance Readings" data forms were not used in the analysis as the initial linear regression equation that was used for these calculations was derived using rounded absorbance values in Excel. All absorbances and concentrations used in SAS calculations and reported within Spectrophotometric Data Summary have been corrected.

File Folder: 11c

Item Number 1
Page 1 of 4

AEH-12-PSEUDO-04

| tank | thero | time | loc | abs | conc |
|---|---|---|---|---|---|
| . | 25 | 0 | . | 0.034 | 25 |
| . | 50 | 0 | . | 0.069 | 50 |
| . | 100 | 0 | . | 0.135 | 100 |
| . | 200 | 0 | . | 0.263 | 200 |
| . | 25 | 0 | . | 0.034 | 25 |
| . | 50 | 0 | . | 0.069 | 50 |
| . | 100 | 0 | . | 0.135 | 100 |
| . | 200 | 0 | . | 0.263 | 200 |
| . | 25 | 0 | . | 0.034 | 25 |
| . | 50 | 0 | . | 0.069 | 50 |
| . | 100 | 0 | . | 0.137 | 100 |
| . | 200 | 0 | . | 0.263 | 200 |
| . | 25 | 6 | . | 0.033 | . |
| . | 50 | 6 | . | 0.068 | . |
| . | 100 | 6 | . | 0.13 | . |
| . | 200 | 6 | . | 0.247 | . |
| . | 25 | 9 | . | 0.033 | . |
| . | 50 | 9 | . | 0.063 | . |
| . | 100 | 9 | . | 0.127 | . |
| . | 200 | 9 | . | 0.248 | . |
| . | 25 | 12 | . | 0.035 | . |
| . | 50 | 12 | . | 0.068 | . |
| . | 100 | 12 | . | 0.129 | . |
| . | 200 | 12 | . | 0.249 | . |
| 1 | 0 | 1 | sur | 0.000 | . |
| 4 | 0 | 1 | sur | 0.001 | . |
| 7 | 0 | 1 | sur | 0.001 | . |
| 2 | 50 | 1 | sur | 0.061 | . |
| 5 | 50 | 1 | sur | 0.072 | . |
| 8 | 50 | 1 | sur | 0.067 | . |
| 3 | 100 | 1 | sur | 0.144 | . |
| 6 | 100 | 1 | sur | 0.150 | . |
| 9 | 100 | 1 | sur | 0.132 | . |
| 1 | 0 | 1 | sus | 0.001 | . |
| 4 | 0 | 1 | sus | 0.000 | . |
| 7 | 0 | 1 | sus | 0.000 | . |
| 2 | 50 | 1 | sus | 0.064 | . |
| 5 | 50 | 1 | sus | 0.073 | . |
| 8 | 50 | 1 | sus | 0.065 | . |
| 3 | 100 | 1 | sus | 0.144 | . |
| 6 | 100 | 1 | sus | 0.149 | . |
| 9 | 100 | 1 | sus | 0.133 | . |
| 1 | 0 | 3 | sur | 0.001 | . |
| 4 | 0 | 3 | sur | 0.003 | . |
| 7 | 0 | 3 | sur | 0.000 | . |
| 2 | 50 | 3 | sur | 0.059 | . |

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| | | | | | |
|---|---|---|---|---|---|
| 5 | 50 | 3 | sur | 0.068 | . |
| 8 | 50 | 3 | sur | 0.063 | . |
| 3 | 100 | 3 | sur | 0.134 | . |
| 6 | 100 | 3 | sur | 0.140 | . |
| 9 | 100 | 3 | sur | 0.124 | . |
| 1 | 0 | 3 | sus | 0.002 | . |
| 4 | 0 | 3 | sus | 0.001 | . |
| 7 | 0 | 3 | sus | 0.000 | . |
| 2 | 50 | 3 | sus | 0.060 | . |
| 5 | 50 | 3 | sus | 0.068 | . |
| 8 | 50 | 3 | sus | 0.062 | . |
| 3 | 100 | 3 | sus | 0.134 | . |
| 6 | 100 | 3 | sus | 0.141 | . |
| 9 | 100 | 3 | sus | 0.124 | . |
| 1 | 0 | 6 | sur | 0.002 | . |
| 4 | 0 | 6 | sur | 0.002 | . |
| 7 | 0 | 6 | sur | 0.001 | . |
| 2 | 50 | 6 | sur | 0.055 | . |
| 5 | 50 | 6 | sur | 0.064 | . |
| 8 | 50 | 6 | sur | 0.058 | . |
| 3 | 100 | 6 | sur | 0.123 | . |
| 6 | 100 | 6 | sur | 0.131 | . |
| 9 | 100 | 6 | sur | 0.116 | . |
| 1 | 0 | 6 | sus | 0.001 | . |
| 4 | 0 | 6 | sus | 0.002 | . |
| 7 | 0 | 6 | sus | 0.003 | . |
| 2 | 50 | 6 | sus | 0.054 | . |
| 5 | 50 | 6 | sus | 0.065 | . |
| 8 | 50 | 6 | sus | 0.060 | . |
| 3 | 100 | 6 | sus | 0.127 | . |
| 6 | 100 | 6 | sus | 0.132 | . |
| 9 | 100 | 6 | sus | 0.115 | . |
| 1 | 0 | 9 | sur | 0.004 | . |
| 4 | 0 | 9 | sur | 0.003 | . |
| 7 | 0 | 9 | sur | 0.005 | . |
| 2 | 50 | 9 | sur | 0.053 | . |
| 5 | 50 | 9 | sur | 0.061 | . |
| 8 | 50 | 9 | sur | 0.056 | . |
| 3 | 100 | 9 | sur | 0.117 | . |
| 6 | 100 | 9 | sur | 0.123 | . |
| 9 | 100 | 9 | sur | 0.108 | . |
| 1 | 0 | 9 | sus | 0.005 | . |
| 4 | 0 | 9 | sus | 0.004 | . |
| 7 | 0 | 9 | sus | 0.004 | . |
| 2 | 50 | 9 | sus | 0.053 | . |
| 5 | 50 | 9 | sus | 0.059 | . |
| 8 | 50 | 9 | sus | 0.055 | . |

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| | | | | | |
|---|---|---|---|---|---|
| 3 | 100 | 9 | sus | 0.119 | . |
| 6 | 100 | 9 | sus | 0.125 | . |
| 9 | 100 | 9 | sus | 0.108 | . |
| 1 | 0 | 12 | sur | 0.005 | . |
| 4 | 0 | 12 | sur | 0.004 | . |
| 7 | 0 | 12 | sur | 0.004 | . |
| 2 | 50 | 12 | sur | 0.051 | . |
| 5 | 50 | 12 | sur | 0.059 | . |
| 8 | 50 | 12 | sur | 0.056 | . |
| 3 | 100 | 12 | sur | 0.113 | . |
| 6 | 100 | 12 | sur | 0.122 | . |
| 9 | 100 | 12 | sur | 0.105 | . |
| 1 | 0 | 12 | sus | 0.006 | . |
| 4 | 0 | 12 | sus | 0.004 | . |
| 7 | 0 | 12 | sus | 0.005 | . |
| 2 | 50 | 12 | sus | 0.053 | . |
| 5 | 50 | 12 | sus | 0.059 | . |
| 8 | 50 | 12 | sus | 0.054 | . |
| 3 | 100 | 12 | sus | 0.115 | . |
| 6 | 100 | 12 | sus | 0.120 | . |
| 9 | 100 | 12 | sus | 0.106 | . |

File Folder: 11c Item Number: 1

```
ods html close; /* close previous */;
ods html; /* open new */;
ods graphics on;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Shawano Lake-whole water treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  26MAR2014  Analysis prepare

/***************************************************************************
* SAS ver 9.3       Analysis prepared by:  JAL      Page ___ of ___
* Analysis completion date:  26MAR2014
***************************************************************************

data Zeq; set shawano.shawanowhole;
run;
proc sort;
by tank time loc; run;

run;
proc gplot data= zeq;
plot abs * conc;
run;
proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
proc sort;
by time tank loc;
proc print data=output_out;
run;
data zeq2; set output_out;
if tank = "." then delete;
if tank = " " then delete;
if loc =" ." then delete;
if time = "0" then delete;
run;
proc sort;
by tank loc;
run;
/***************************************************************************
```

FF # 11c
Item No. 2
Pg 1 of 4

```
 * This procedure produces the mean concentrations for each treatment replicat
 * by the the sampling location
 * i.e. It gives the mean concentration of each treatment tank over the entire
 * location [i.e. surface vs suspended sampling
 *****************************************************************************
title "Mean treatment concentration by treatment tank and sampling location (s
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;
proc sort;
by thero time loc;
/*****************************************************************************
 * This procedure produces the mean concentrations for each treatment group ov
 * by the sampling location
 * i.e. It gives the mean concentration of the 3 control, 50ppm & 100ppm treat
 * sampling location (surface/suspended) over the entire exposure
 *****************************************************************************
title "Mean treatment concentration by treatment group and sampling location f
proc sort;
by thero loc time;

proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;
data time9; set zeq2;
if time > 9 then delete;
run;
proc sort;
by thero loc time;
/*****************************************************************************
 * This procedure produces the mean concentrations for each treatment group th
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks through th
 *****************************************************************************
title "Mean treatment concentration by treatment group and sampling location t

proc means data = time9 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

data time6; set zeq2;
if time > 6 then delete;
run;
```

```
proc sort;
by thero loc time;

/*****************************************************************************
 * This procedure produces the mean concentrations for each treatment group th
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks through th
 *****************************************************************************
title "Mean treatment concentration by treatment group and sampling location t

proc means data = time6 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;


/*****************************************************************************
 * This procedure produces the mean concentrations for each treatment group by
 * i.e. It gives the mean conc. of the 3 control,50ppm & 100ppm  treatment tar
 *****************************************************************************
title "Mean treatment concentration for each treatment group for each sampling

proc sort;
by thero loc time;


proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc time;
var predicted_ppm;
run;

data zeq3; set output_out;
if conc > 1 then delete;
if tank > 0.5 then delete;
if thero = "." then delete;
run;

proc sort;
by thero;
/*****************************************************************************
 * This procedure produces the mean concentrations for the standard checks for
 * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at 6, 9
 *****************************************************************************
title "Mean concentration for standard checks for all sampling times';
proc means data = zeq3 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
```

3-26-14
JW

```
run;
quit;
run;
```

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FF # 11C
Item No. 2
Pg 4 of 4

```
4    DM  LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
5
6    FOOTNOTE1 'Performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE
6  ! ;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.
7
8    options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;
9
10   title1 h=1 'Standard Curve Linear Regression and sample concentrations';
11   title2 h=1 'Study # AEH-12-PSUEDO-04';
12   title3 h=1 'Shawano Lake-whole water treatment';
13   title4 h=1 'SAS v. 9.3  Analysis completion date:  26MAR2014  Analysis pr
14
15   /***********************************************************************
16   * SAS ver 9.3       Analysis prepared by:  JAL        Page ___ of ___
17   * Analysis completion date:  26MAR2014   JAL
18   ***********************************************************************
19
20   data Zeq; set shawano.shawanowhole;
21   run;

NOTE: There were 115 observations read from the data set SHAWANO.SHAWANOWHOLE.
NOTE: The data set WORK.ZEQ has 115 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.03 seconds


22   proc sort;
23   by tank time loc; run;

NOTE: There were 115 observations read from the data set WORK.ZEQ.
NOTE: The data set WORK.ZEQ has 115 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


24
25   run;
26   proc gplot data= zeq;
27   plot abs * conc;
28   run;

NOTE: 103 observation(s) contained a MISSING value for the abs * conc request.
```

FF # 11C
Item No. 3
Pg 1 of 8

```
NOTE: 4 records written to C:\Users\klweber\gplot.png.

NOTE: There were 115 observations read from the data set WORK.ZEQ.
NOTE: PROCEDURE GPLOT used (Total process time):
      real time           0.65 seconds
      cpu time            0.49 seconds


proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;

NOTE: The data set WORK.OUTPUT_OUT has 115 observations and 7 variables.
NOTE: PROCEDURE REG used (Total process time):
      real time           4.49 seconds
      cpu time            0.63 seconds


proc sort;
by time tank loc;

NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.OUTPUT_OUT has 115 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data=output_out;
run;

NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.15 seconds
      cpu time            0.07 seconds


data zeq2; set output_out;
if tank = "." then delete;
if tank = " " then delete;
if loc ="." then delete;
if time = "0" then delete;
run;
```

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```
NOTE: Character values have been converted to numeric values at the places gi\
      (Line):(Column).
      41:11
NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ2 has 90 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by tank loc;
run;

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 90 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


/*************************************************************************
**************
  * This procedure produces the mean concentrations for each treatment rep
sampling times  *
  * by the the sampling location
            *
  * i.e. It gives the mean concentration of each treatment tank over the e
the sampling *
  * location [i.e. surface vs suspended sanpling
            *
  ************************************************************************
**************/
title "Mean treatment concentration by treatment tank and sampling locati
(surface/suspended) for all sampling times";
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.15 seconds
      cpu time            0.06 seconds
```

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```
proc so~t;
by thero time loc;
/***********************************************************************
! **************
 * This procedure produces the mean concentrations for each treatment grc
! sampling times      *
 * by the sampling location
!             *
 * i.e. It gives the mean concentration of the 3 control, 50ppm & 100ppm
!             *
 * sampling location (surface/suspended) over the entire exposure
!             *
***********************************************************************
! **************/
title "Mean treatment concentration by treatment group and sampling locat
! sampling times";

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 90 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by thero loc time;


NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 90 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.01 seconds
```

```
data time9; set zeq2;
if time > 9 then delete;
run;

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.TIME9 has 72 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by thero loc time;
/************************************************************************
********************
 * This procedure produces the mean concentrations for each treatment grc
sample location       *
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks throu
 by sample location *
 ************************************************************************
********************/
title "Mean treatment concentration by treatment group and sampling locat
 exposure";


NOTE: There were 72 observations read from the data set WORK.TIME9.
NOTE: The data set WORK.TIME9 has 72 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc means data = time9 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

NOTE: There were 72 observations read from the data set WORK.TIME9.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.01 seconds



data time6; set zeq2;
if time > 6 then delete;
```

```
run;

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.TIME6 has 54 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc sort;
by thero loc time;

/*************************************************************************
*********************
 * This procedure produces the mean concentrations for each treatment grc
sample location      *
 * i.e. Mean concentration of the 3 50ppm & 100ppm  treatment tanks throu
 by sample location *
 ************************************************************************
*********************/
title "Mean treatment concentration by treatment group and sampling locat
 exposure";

NOTE: There were 54 observations read from the data set WORK.TIME6.
NOTE: The data set WORK.TIME6 has 54 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc means data = time6 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;

NOTE: There were 54 observations read from the data set WORK.TIME6.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.14 seconds
      cpu time            0.03 seconds


/*************************************************************************
```

```
108!  *********************
109    * This procedure produces the mean concentrations for each treatment grc
109!                       *
110    * i.e. It gives the mean conc. of the 3 control,50ppm & 100ppm  treatmer
110!  3, 6, 9 and 12h      *
111    ***********************************************************************
111!  *********************/
112   title "Mean treatment concentration for each treatment group for each sam
113
114   proc sort;
115   by thero loc time;
116
117

NOTE: Input data set is already sorted, no sorting done.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


118   proc means data = zeq2 mean std lclm uclm fw=8;
119   by thero loc time;
120   var predicted_ppm;
121   run;

NOTE: There were 90 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.15 seconds
      cpu time            0.07 seconds


122
123   data zeq3; set output_out;
124   if conc > 1 then delete;
125   if tank > 0.5 then delete;
126   if thero = "." then delete;
127   run;

NOTE: Character values have been converted to numeric values at the places giv
      (Line):(Column).
      125:4    126:12
NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ3 has 12 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds
```

```

proc sort;
by thero;
/*************************************************************************
**************
 * This procedure produces the mean concentrations for the standard check
periods        *
 * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at
          *
*************************************************************************
**************/
title "Mean concentration for standard checks for all sampling times";

NOTE: There were 12 observations read from the data set WORK.ZEQ3.
NOTE: The data set WORK.ZEQ3 has 12 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds

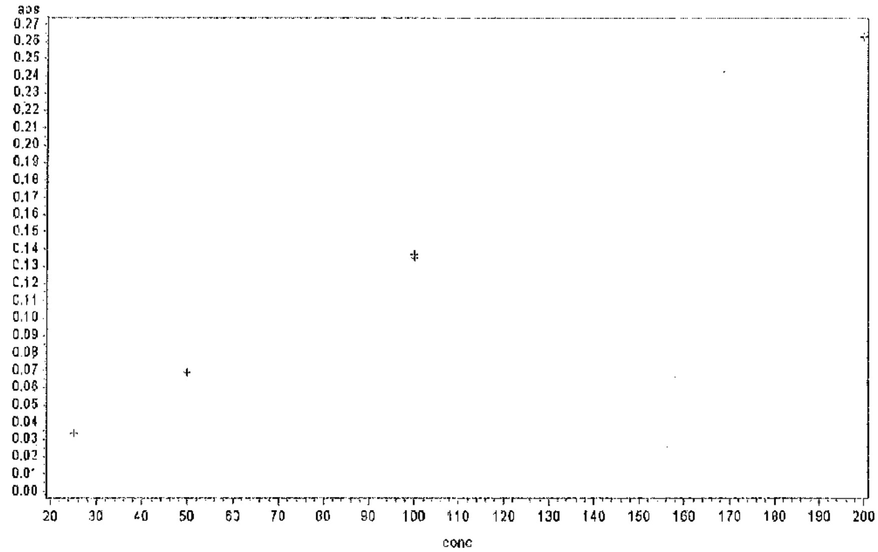

proc means data = zeq3 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
run;

NOTE: There were 12 observations read from the data set WORK.ZEQ3.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.10 seconds
      cpu time            0.03 seconds


quit;
run;
```

AEH-12-PSEUDO-04

Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-whole water treatment
SAS v. 9.3 Analysis completion date: 20MAR2014 Analysis prepared by: JAL JL




Performed by J. Luoma SAS version 9.3 07:40 26MAR14

FF # 11C
Item No. 4
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**Standard Curve Linear Regression and sample concentrations**
**Study # AEH-12-PSUEDO-04**
**Shawano Lake-whole water treatment**
**SAS v. 9.3 Analysis completion date: 26MAR2014 Analysis prepared by: JAL**

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc

| | |
|---|---|
| **Number of Observations Read** | 115 |
| **Number of Observations Used** | 12 |
| **Number of Observations with Missing Values** | 103 |

**Analysis of Variance**

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 1 | 53889 | 53889 | 31334.2 | <.0001 |
| **Error** | 10 | 17.19816 | 1.71982 | | |
| **Corrected Total** | 11 | 53906 | | | |

| | | | |
|---|---|---|---|
| **Root MSE** | 1.31142 | **R-Square** | 0.9997 |
| **Dependent Mean** | 93.75000 | **Adj R-Sq** | 0.9996 |
| **Coeff Var** | 1.39885 | | |

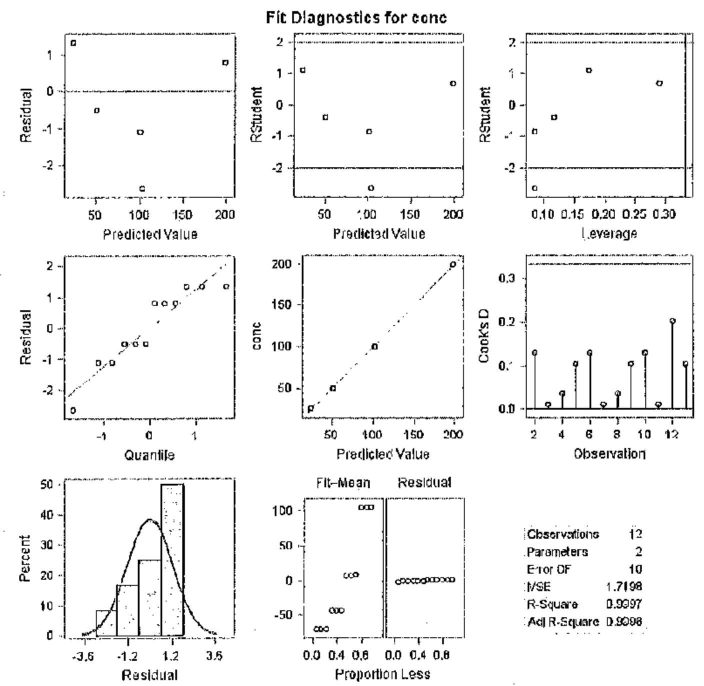
**Parameter Estimates**

| Variable | Label | DF | Parameter Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| **Intercept** | Intercept | 1 | -2.38065 | 0.66200 | -3.60 | 0.0049 |
| **abs** | abs | 1 | 766.49020 | 4.33009 | 177.01 | <.0001 |

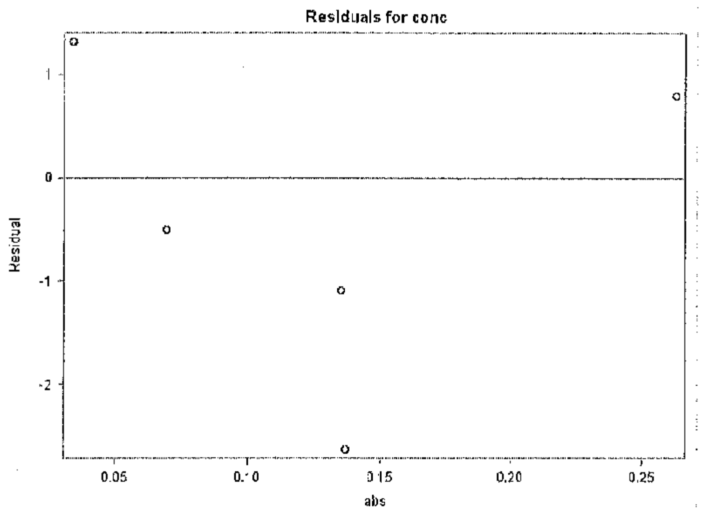
Performed by J. Luoma SAS version 9.3 07:40 26MAR14

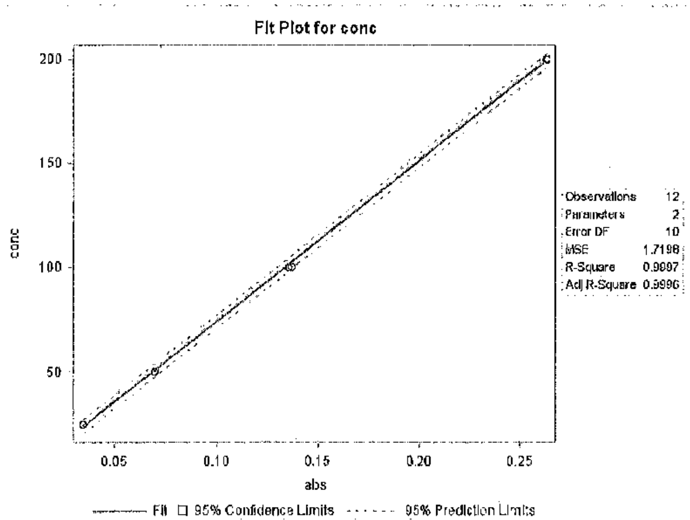
Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-whole water treatment
SAS v. 9.3 Analysis completion date: 26MAR2014 Analysis prepared by: JAL

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc



AEH-12-PSEUDO-04




Performed by J. Luoma SAS version 9.3 07:40 26MAR14

AEH-12-PSEUDO-04

Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-whole water treatment
SAS v. 9.3 Analysis completion date: 26MAR2014 Analysis prepared by: JAL

| Obs | tank | thero | time | loc | abs | conc | predicted_ppm |
|---|---|---|---|---|---|---|---|
| 1 | | . | . | | . | . | . |
| 2 | . | 25 | 0 | . | 0.034 | 25 | 23.680 |
| 3 | . | 50 | 0 | . | 0.069 | 50 | 50.507 |
| 4 | . | 100 | 0 | . | 0.135 | 100 | 101.096 |
| 5 | . | 200 | 0 | . | 0.263 | 200 | 199.206 |
| 6 | . | 25 | 0 | . | 0.034 | 25 | 23.680 |
| 7 | . | 50 | 0 | . | 0.069 | 50 | 50.507 |
| 8 | . | 100 | 0 | . | 0.135 | 100 | 101.096 |
| 9 | . | 200 | 0 | . | 0.263 | 200 | 199.206 |
| 10 | . | 25 | 0 | . | 0.034 | 25 | 23.680 |
| 11 | . | 50 | 0 | . | 0.069 | 50 | 50.507 |
| 12 | . | 100 | 0 | . | 0.137 | 100 | 102.629 |
| 13 | . | 200 | 0 | . | 0.263 | 200 | 199.206 |
| 14 | 1 | 0 | 1 | sur | 0.000 | . | -2.381 |
| 15 | 1 | 0 | 1 | sus | 0.001 | . | -1.614 |
| 16 | 2 | 50 | 1 | sur | 0.061 | . | 44.375 |
| 17 | 2 | 50 | 1 | sus | 0.064 | . | 46.675 |
| 18 | 3 | 100 | 1 | sur | 0.144 | . | 107.994 |
| 19 | 3 | 100 | 1 | sus | 0.144 | . | 107.994 |
| 20 | 4 | 0 | 1 | sur | 0.001 | . | -1.614 |
| 21 | 4 | 0 | 1 | sus | 0.000 | . | -2.381 |
| 22 | 5 | 50 | 1 | sur | 0.072 | . | 52.807 |
| 23 | 5 | 50 | 1 | sus | 0.073 | . | 53.573 |
| 24 | 6 | 100 | 1 | sur | 0.150 | . | 112.593 |
| 25 | 6 | 100 | 1 | sus | 0.149 | . | 111.826 |
| 26 | 7 | 0 | 1 | sur | 0.001 | . | -1.614 |
| 27 | 7 | 0 | 1 | sus | 0.000 | . | -2.381 |
| 28 | 8 | 50 | 1 | sur | 0.067 | . | 48.974 |
| 29 | 8 | 50 | 1 | sus | 0.065 | . | 47.441 |
| 30 | 9 | 100 | 1 | sur | 0.132 | . | 98.796 |
| 31 | 9 | 100 | 1 | sus | 0.133 | . | 99.563 |
| 32 | 1 | 0 | 3 | sur | 0.001 | . | -1.614 |
| 33 | 1 | 0 | 3 | sus | 0.002 | . | -0.848 |
| 34 | 2 | 50 | 3 | sur | 0.059 | . | 42.842 |
| 35 | 2 | 50 | 3 | sus | 0.060 | . | 43.609 |
| 36 | 3 | 100 | 3 | sur | 0.134 | . | 100.329 |
| 37 | 3 | 100 | 3 | sus | 0.134 | . | 100.329 |
| 38 | 4 | 0 | 3 | sur | 0.003 | . | -0.081 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 39 | 4 | 0 | 3 | sus | 0.001 | . | -1.614 |
| 40 | 5 | 50 | 3 | sur | 0.068 | . | 49.741 |
| 41 | 5 | 50 | 3 | sus | 0.068 | . | 49.741 |
| 42 | 6 | 100 | 3 | sur | 0.140 | . | 104.928 |
| 43 | 6 | 100 | 3 | sus | 0.141 | . | 105.694 |
| 44 | 7 | 0 | 3 | sur | 0.000 | . | -2.381 |
| 45 | 7 | 0 | 3 | sus | 0.000 | . | -2.381 |
| 46 | 8 | 50 | 3 | sur | 0.063 | . | 45.908 |
| 47 | 8 | 50 | 3 | sus | 0.062 | . | 45.142 |
| 48 | 9 | 100 | 3 | sur | 0.124 | . | 92.664 |
| 49 | 9 | 100 | 3 | sus | 0.124 | . | 92.664 |
| 50 | . | 25 | 6 | . | 0.033 | . | 22.914 |
| 51 | . | 50 | 6 | . | 0.068 | . | 49.741 |
| 52 | . | 100 | 6 | . | 0.130 | . | 97.263 |
| 53 | . | 200 | 6 | . | 0.247 | . | 186.942 |
| 54 | 1 | 0 | 6 | sur | 0.002 | . | -0.848 |
| 55 | 1 | 0 | 6 | sus | 0.001 | . | -1.614 |
| 56 | 2 | 50 | 6 | sur | 0.055 | . | 39.776 |
| 57 | 2 | 50 | 6 | sus | 0.054 | . | 39.010 |
| 58 | 3 | 100 | 6 | sur | 0.123 | . | 91.898 |
| 59 | 3 | 100 | 6 | sus | 0.127 | . | 94.964 |
| 60 | 4 | 0 | 6 | sur | 0.002 | . | -0.848 |
| 61 | 4 | 0 | 6 | sus | 0.002 | . | -0.848 |
| 62 | 5 | 50 | 6 | sur | 0.064 | . | 46.675 |
| 63 | 5 | 50 | 6 | sus | 0.065 | . | 47.441 |
| 64 | 6 | 100 | 6 | sur | 0.131 | . | 98.030 |
| 65 | 6 | 100 | 6 | sus | 0.132 | . | 98.796 |
| 66 | 7 | 0 | 6 | sur | 0.001 | . | -1.614 |
| 67 | 7 | 0 | 6 | sus | 0.003 | . | -0.081 |
| 68 | 8 | 50 | 6 | sur | 0.058 | . | 42.076 |
| 69 | 8 | 50 | 6 | sus | 0.060 | . | 43.609 |
| 70 | 9 | 100 | 6 | sur | 0.116 | . | 86.532 |
| 71 | 9 | 100 | 6 | sus | 0.115 | . | 85.766 |
| 72 | . | 25 | 9 | . | 0.033 | . | 22.914 |
| 73 | . | 50 | 9 | . | 0.063 | . | 45.908 |
| 74 | . | 100 | 9 | . | 0.127 | . | 94.964 |
| 75 | . | 200 | 9 | . | 0.248 | . | 187.709 |
| 76 | 1 | 0 | 9 | sur | 0.004 | . | 0.685 |
| 77 | 1 | 0 | 9 | sus | 0.005 | . | 1.452 |
| 78 | 2 | 50 | 9 | sur | 0.053 | . | 38.243 |
| 79 | 2 | 50 | 9 | sus | 0.053 | . | 38.243 |
| 80 | 3 | 100 | 9 | sur | 0.117 | . | 87.299 |
| 81 | | | | | | | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 100 | 9 | sus | 0.119 | . | 88.832 |
| 82 | 4 | 0 | 9 | sur | 0.003 | . | -0.081 |
| 83 | 4 | 0 | 9 | sus | 0.004 | . | 0.685 |
| 84 | 5 | 50 | 9 | sur | 0.061 | . | 44.375 |
| 85 | 5 | 50 | 9 | sus | 0.059 | . | 42.842 |
| 86 | 6 | 100 | 9 | sur | 0.123 | . | 91.898 |
| 87 | 6 | 100 | 9 | sus | 0.125 | . | 93.431 |
| 88 | 7 | 0 | 9 | sur | 0.005 | . | 1.452 |
| 89 | 7 | 0 | 9 | sus | 0.004 | . | 0.685 |
| 90 | 8 | 50 | 9 | sur | 0.056 | . | 40.543 |
| 91 | 8 | 50 | 9 | sus | 0.055 | . | 39.776 |
| 92 | 9 | 100 | 9 | sur | 0.108 | . | 80.400 |
| 93 | 9 | 100 | 9 | sus | 0.108 | . | 80.400 |
| 94 | . | 25 | 12 | . | 0.035 | . | 24.447 |
| 95 | . | 50 | 12 | . | 0.068 | . | 49.741 |
| 96 | . | 100 | 12 | . | 0.129 | . | 96.497 |
| 97 | . | 200 | 12 | . | 0.249 | . | 188.475 |
| 98 | 1 | 0 | 12 | sur | 0.005 | . | 1.452 |
| 99 | 1 | 0 | 12 | sus | 0.006 | . | 2.218 |
| 100 | 2 | 50 | 12 | sur | 0.051 | . | 36.710 |
| 101 | 2 | 50 | 12 | sus | 0.053 | . | 38.243 |
| 102 | 3 | 100 | 12 | sur | 0.113 | . | 84.233 |
| 103 | 3 | 100 | 12 | sus | 0.115 | . | 85.766 |
| 104 | 4 | 0 | 12 | sur | 0.004 | . | 0.685 |
| 105 | 4 | 0 | 12 | sus | 0.004 | . | 0.685 |
| 106 | 5 | 50 | 12 | sur | 0.059 | . | 42.842 |
| 107 | 5 | 50 | 12 | sus | 0.059 | . | 42.842 |
| 108 | 6 | 100 | 12 | sur | 0.122 | . | 91.131 |
| 109 | 6 | 100 | 12 | sus | 0.120 | . | 89.598 |
| 110 | 7 | 0 | 12 | sur | 0.004 | . | 0.685 |
| 111 | 7 | 0 | 12 | sus | 0.005 | . | 1.452 |
| 112 | 8 | 50 | 12 | sur | 0.056 | . | 40.543 |
| 113 | 8 | 50 | 12 | sus | 0.054 | . | 39.010 |
| 114 | 9 | 100 | 12 | sur | 0.105 | . | 78.101 |
| 115 | 9 | 100 | 12 | sus | 0.106 | . | 78.867 |

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**Mean treatment concentration by treatment tank and sampling location (surface/suspended) for all sampling times**

The MEANS Procedure

tank=1 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.5411 | 1.5894 | -2.5146 | 1.4325 |

tank=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.0812 | 1.7976 | -2.3132 | 2.1508 |

tank=2 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 40.3895 | 3.1789 | 36.4424 | 44.3366 |

tank=2 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 41.1560 | 3.8094 | 36.4260 | 45.8860 |

tank=3 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 94.3504 | 9.7468 | 82.2482 | 106.5 |

tank=3 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 95.5768 | 8.9289 | 84.4901 | 106.7 |

tank=4 loc=sur

Analysis Variable : predicted_ppm Predicted

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| Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.3878 | 0.8739 | -1.4729 | 0.6974 |

tank=4 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.6944 | 1.3711 | -2.3969 | 1.0081 |

tank=5 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 47.2879 | 4.0341 | 42.2789 | 52.2969 |

tank=5 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 47.2879 | 4.6117 | 41.5617 | 53.0141 |

tank=6 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.7158 | 9.0919 | 88.4268 | 111.0 |

tank=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.8691 | 9.0140 | 88.6768 | 111.1 |

tank=7 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.6944 | 1.6617 | -2.7577 | 1.3689 |

tank=7 loc=sus

Analysis Variable : predicted_ppm Predicted

| Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.5411 | 1.7646 | -2.7321 | 1.6500 |

tank=8 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.6088 | 3.7157 | 38.9951 | 48.2224 |

tank=8 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 42.9956 | 3.5706 | 38.5621 | 47.4290 |

tank=9 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 87.2987 | 8.5696 | 76.6581 | 97.9393 |

tank=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 87.4520 | 8.6617 | 76.6971 | 98.2069 |

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**Mean treatment concentration by treatment group and sampling location for all sampling times**

The MEANS Procedure

**thero=0 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.5411 | 1.3213 | -1.2728 | 0.1906 |

**thero=0 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.4389 | 1.5565 | -1.3008 | 0.4231 |

**thero=50 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.7621 | 4.4712 | 41.2860 | 46.2382 |

**thero=50 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.8132 | 4.5758 | 41.2792 | 46.3471 |

**thero=100 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 93.7883 | 9.9722 | 88.2659 | 99.3108 |

**thero=100 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 94.2993 | 9.7896 | 88.8780 | 99.7206 |

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**Mean treatment concentration by treatment group and sampling location through the 9 h exposure**

**The MEANS Procedure**

**thero=0 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.9115 | 1.1990 | -1.6734 | -0.1497 |

**thero=0 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.9115 | 1.3259 | -1.7540 | -0.0691 |

**thero=50 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 44.6946 | 4.3539 | 41.9283 | 47.4609 |

**thero=50 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 44.7585 | 4.5464 | 41.8699 | 47.6471 |

**thero=100 loc=sur**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 96.1133 | 9.4528 | 90.1074 | 102.1 |

**thero=100 loc=sus**

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 96.6882 | 9.2449 | 90.8143 | 102.6 |

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**Mean treatment concentration by treatment group and sampling location through the 6 h exposure**

The MEANS Procedure

thero=0 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.4438 | 0.7449 | -2.0164 | -0.8712 |

thero=0 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.5290 | 0.8080 | -2.1500 | -0.9079 |

thero=50 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 45.9082 | 4.1277 | 42.7354 | 49.0810 |

thero=50 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 46.2489 | 4.1297 | 43.0746 | 49.4232 |

thero=100 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.3071 | 8.2820 | 92.9409 | 105.7 |

thero=100 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.7329 | 8.0552 | 93.5411 | 105.9 |

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Mean treatment concentration for each treatment group for each sampling time

The MEANS Procedure

thero=0 loc=sur time=1

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -1.8697 | 0.4425 | -2.9690 | -0.7703 |

thero=0 loc=sur time=3

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -1.3587 | 1.1708 | -4.2672 | 1.5499 |

thero=0 loc=sur time=6

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -1.1032 | 0.4425 | -2.2025 | -0.00385 |

thero=0 loc=sur time=9

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 0.6853 | 0.7665 | -1.2188 | 2.5894 |

thero=0 loc=sur time=12

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 0.9408 | 0.4425 | -0.1585 | 2.0401 |

thero=0 loc=sus time=1

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -2.1251 | 0.4425 | -3.2245 | -1.0258 |

thero=0 loc=sus time=3

Analysis Variable : predicted_ppm Predicted Value of conc

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -1.6142 | 0.7665 | -3.5182 | 0.2899 |

thero=0 loc=sus time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.8477 | 0.7665 | -2.7517 | 1.0564 |

thero=0 loc=sus time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 0.9408 | 0.4425 | -0.1585 | 2.0401 |

thero=0 loc=sus time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 1.4518 | 0.7665 | -0.4523 | 3.3559 |

thero=50 loc=sur time=1

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 48.7187 | 4.2215 | 38.2319 | 59.2055 |

thero=50 loc=sur time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 46.1637 | 3.4563 | 37.5778 | 54.7496 |

thero=50 loc=sur time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 42.8423 | 3.5125 | 34.1167 | 51.5678 |

thero=50 loc=sur time=9

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 41.0538 | 3.0977 | 33.3586 | 48.7490 |

thero=50 loc=sur time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 40.0318 | 3.0977 | 32.3366 | 47.7270 |

thero=50 loc=sus time=1

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 49.2297 | 3.7810 | 39.8372 | 58.6222 |

thero=50 loc=sus time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 46.1637 | 3.1912 | 38.2365 | 54.0910 |

thero=50 loc=sus time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 43.3533 | 4.2215 | 32.8665 | 53.8401 |

thero=50 loc=sus time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 40.2873 | 2.3417 | 34.4703 | 46.1043 |

thero=50 loc=sus time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 40.0318 | 2.4639 | 33.9111 | 46.1525 |

thero=100 loc=sur time=1

Analysis Variable : predicted_ppm Predicted Value of conc

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 106.5 | 7.0250 | 89.0099 | 123.9 |

thero=100 loc=sur time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.3071 | 6.1955 | 83.9167 | 114.7 |

thero=100 loc=sur time=6

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 92.1531 | 5.7529 | 77.8621 | 106.4 |

thero=100 loc=sur time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 86.5322 | 5.7869 | 72.1568 | 100.9 |

thero=100 loc=sur time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 84.4882 | 6.5189 | 68.2943 | 100.7 |

thero=100 loc=sus time=1

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 106.5 | 6.2740 | 90.8755 | 122.0 |

thero=100 loc=sus time=3

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.5626 | 6.5489 | 83.2942 | 115.8 |

thero=100 loc=sus time=6

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 93.1751 | 6.6967 | 76.5395 | 109.8 |

thero=100 loc=sus time=9

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 87.5542 | 6.6084 | 71.1379 | 104.0 |

thero=100 loc=sus time=12

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 84.7437 | 5.4379 | 71.2351 | 98.2523 |

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Mean concentration for standard checks for all sampling times

The MEANS Procedure

thero=25

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 23.4245 | 0.8851 | 21.2259 | 25.6232 |

thero=50

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 48.4632 | 2.2127 | 42.9666 | 53.9598 |

thero=100

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 96.2411 | 1.1708 | 93.3326 | 99.1496 |

thero=200

Analysis Variable : predicted_ppm Predicted Value of conc

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 187.7 | 0.7665 | 185.8 | 189.6 |

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FF # 11C
Item No. 4
Pg 19 of 19

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 14 - 17)
Data Source: File Folder: 12c
Forms: "Sample Absorbance Readings" Data Sheet

File Name: See filenames as stated below

| Action | Date | Initials |
|---|---|---|
| Created...... | 6-Feb-14 | TJS TJS |
| Revised...... | 12-Feb-14 | TJS TJS |
| Reviewed... | 12-FEB14 | TJS |
| Certified..... | 2/15/14 | SAW |

**Spectrophotometric Data**

Test Article: Zequanox ® (MBI-401 SDP)
Test Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

**Data Explanation:**

1) The absorbance of triplicate samples of 25, 50, 100, and 200 mg/L dilutions of a 2,000 mg/L active ingredient (A.I.) stock prepared from Analytical Stock #2 were measured to prepare a standard curve.
2) Standard checks were performed at 6, 9, and 12 hours by comparing the 25, 50, 100, and 200 mg/L (A.I.) dilutions to the linear curve.
3) Data codes used within SAS
- tank = Tank ID (1 through 9)
- thero = Theoretical or target concentration (mg/L)
- time = Sample Time (0, 1, 3, 6, 9, and 12 h after treatment)
- loc = Sample Location
  - sus = Suspended Sample (sampled ~19 cm from bottom of tank)
  - bot = Bottom Sample (sampled from bottom of tank)
- abs = measured absorbance of sample
- conc = concentration ([mg/L], only used for standards used for regression)

4) Information that is not relevant to a sample (i.e., tank ID for standards) or that will be calculated by SAS (i.e., predicted concentration for standard checks and samples) is denoted by a "." in the SAS input and output files.

**Data Analysis:**

1) A linear regression was completed in SAS using the absorbance values obtained from the spectrophotometer of 3 replicate dilutions of 25, 50, 100 and 200 mg/L Zequanox
2) Standard checks and treatment sample concentrations were predicted in SAS by comparing the observed absorbances with the linear regression.
3) The following mean treatment concentrations were determined in SAS:
- 3a) Mean (standard deviation) concentration by tank and location for all sampling times
- 3b) Mean (standard deviation) concentration by treatment group and location for all sampling times
- 3c) Mean (standard deviation) concentration by treatment group and sampling times for both locations
- 3d) Mean (standard deviation) concentrations for 25, 50, 100, and 200 mg/L (A.I.) dilutions for all sampling times

**File Names:**

Spectrophotometric Data for SAS input
I:\AEH-12-PSEUDO-04\Data Summaries\spec\[Lake Carlos Bottom Injection Spec Summary.xlsx]Spec Data for SAS

SAS Program/Code
I:\AEH-12-PSEUDO-04\SAS-Spec\carlos injection program file

SAS Log
I:\AEH-12-PSEUDO-04\SAS-Spec\carlos injection log file

SAS Output
I:\AEH-12-PSEUDO-04\SAS-Spec\carlos injection results file

**Data Anomalies and Deviations:**

1) One exposure tank of a different concentration and sample location was sampled in triplicate to evaluate variability of spectrophotometer during each sampling time. The mean of the triplicates was used within the analysis.
2) Suspended samples (sus) were collected ~19 cm from the tank bottoms for all sampling times; tank bottom (bot) samples were collected at 6, 9 and 12 h sampling times. Test article settling was observed in the 1 and 3 h samples resulting in lower than expected concentrations. Samples collected from the tank bottoms at 6, 9 and 12 h confirmed test article settling as indicated by higher than expected concentrations.
3) Some mean absorbances for triplicate samples may be recorded incorrectly on "Sample Absorbance Readings" data forms as proper significant figure rules may not have been observed. Additionally, concentrations recorded on "Sample Absorbance Readings" data forms were not used in the analysis as the initial linear regression equation that was used for these calculations was derived using rounded absorbance values in Excel. All absorbances and concentrations used in SAS calculations and reported within Spectrophotometric Data Summary have been corrected.

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File Folder: 12c

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| tank | thero | time | loc | abs | conc |
|---|---|---|---|---|---|
| . | 25 | 0 | . | 0.039 | 25 |
| . | 50 | 0 | . | 0.076 | 50 |
| . | 100 | 0 | . | 0.151 | 100 |
| . | 200 | 0 | . | 0.295 | 200 |
| . | 25 | 0 | . | 0.039 | 25 |
| . | 50 | 0 | . | 0.075 | 50 |
| . | 100 | 0 | . | 0.150 | 100 |
| . | 200 | 0 | . | 0.294 | 200 |
| . | 25 | 0 | . | 0.039 | 25 |
| . | 50 | 0 | . | 0.074 | 50 |
| . | 100 | 0 | . | 0.150 | 100 |
| . | 200 | 0 | . | 0.295 | 200 |
| . | 25 | 6 | . | 0.036 | . |
| . | 50 | 6 | . | 0.065 | . |
| . | 100 | 6 | . | 0.135 | . |
| . | 200 | 6 | . | 0.270 | . |
| . | 25 | 9 | . | 0.033 | . |
| . | 50 | 9 | . | 0.069 | . |
| . | 100 | 9 | . | 0.135 | . |
| . | 200 | 9 | . | 0.265 | . |
| . | 25 | 12 | . | 0.034 | . |
| . | 50 | 12 | . | 0.068 | . |
| . | 100 | 12 | . | 0.135 | . |
| . | 200 | 12 | . | 0.265 | . |
| 1 | 50 | 1 | sus | 0.000 | . |
| 4 | 50 | 1 | sus | 0.002 | . |
| 8 | 50 | 1 | sus | 0.001 | . |
| 2 | 100 | 1 | sus | 0.009 | . |
| 5 | 100 | 1 | sus | 0.003 | . |
| 9 | 100 | 1 | sus | 0.036 | . |
| 1 | 50 | 3 | sus | 0.001 | . |
| 4 | 50 | 3 | sus | 0.001 | . |
| 8 | 50 | 3 | sus | 0.000 | . |
| 2 | 100 | 3 | sus | 0.001 | . |
| 5 | 100 | 3 | sus | 0.002 | . |
| 9 | 100 | 3 | sus | 0.000 | . |
| 1 | 50 | 6 | sus | 0.005 | . |
| 4 | 50 | 6 | sus | 0.005 | . |
| 8 | 50 | 6 | sus | 0.001 | . |
| 2 | 100 | 6 | sus | 0.006 | . |
| 5 | 100 | 6 | sus | 0.001 | . |
| 9 | 100 | 6 | sus | 0.000 | . |
| 1 | 50 | 6 | bot | 0.171 | . |
| 4 | 50 | 6 | bot | 0.187 | . |
| 8 | 50 | 6 | bot | 0.207 | . |
| 2 | 100 | 6 | bot | 0.430 | . |

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| | | | | | |
|---|---|---|---|---|---|
| 5 | 100 | 6 | bot | 0.398 | . |
| 9 | 100 | 6 | bot | 0.345 | . |
| 1 | 50 | 9 | sus | 0.004 | . |
| 4 | 50 | 9 | sus | 0.001 | . |
| 8 | 50 | 9 | sus | 0.003 | . |
| 2 | 100 | 9 | sus | 0.006 | . |
| 5 | 100 | 9 | sus | 0.001 | . |
| 9 | 100 | 9 | sus | 0.003 | . |
| 1 | 50 | 9 | bot | 0.159 | . |
| 4 | 50 | 9 | bot | 0.154 | . |
| 8 | 50 | 9 | bot | 0.131 | . |
| 2 | 100 | 9 | bot | 0.395 | . |
| 5 | 100 | 9 | bot | 0.357 | . |
| 9 | 100 | 9 | bot | 0.322 | . |
| 1 | 50 | 12 | sus | 0.011 | . |
| 4 | 50 | 12 | sus | 0.012 | . |
| 8 | 50 | 12 | sus | 0.013 | . |
| 2 | 100 | 12 | sus | 0.004 | . |
| 5 | 100 | 12 | sus | 0.005 | . |
| 9 | 100 | 12 | sus | 0.012 | . |
| 1 | 50 | 12 | bot | 0.108 | . |
| 4 | 50 | 12 | bot | 0.113 | . |
| 8 | 50 | 12 | bot | 0.114 | . |
| 2 | 100 | 12 | bot | 0.324 | . |
| 5 | 100 | 12 | bot | 0.285 | . |
| 9 | 100 | 12 | bot | 0.257 | . |

```
ods html close; /* close previous */;
ods html; /* open new */;
ods graphics on;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
```

AEH-12-PSEUDO-04

```
FOOTNOTE1 'Performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Lake Carlos-injection treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  30Jan2014  Analysis prepared by: JAL';

/***************************************************************************
* SAS ver 9.3       Analysis prepared by:  JAL        Page ___ of ___        *
* Analysis completion date:  30Jan2014  Jn                                   *
***************************************************************************/

data Zeq; set carlos.carlosinjection;
run;
proc sort;
by tank time loc; run;

run;
proc gplot data= zeq;
plot abs * conc;
run;
proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
proc sort;
by time tank loc;
proc print data=output_out;
run;
data zeq2; set output_out;
if tank = "." then delete;
if loc =". " then delete;
if time = "0" then delete;
run;
proc sort;
by tank loc;
run;
/**********************************************************************************************
 * This procedure produces the mean concentrations for each treatment replicate over all sampling tim
 * by the the sampling location
 * i.e. It gives the mean concentration of each treatment tank over the entire exposure by  the sampl
 * location [i.e. bottom vs suspended sampling (bottom sampling initiated at 6h)]
 **********************************************************************************************
title 'Mean treatment concentration by treatment tank and sampling location (bottom/suspended) for al
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;
proc sort;
```

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```
by thero time loc;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for each treatment group over all sampling times
 * by the sampling location
 * i.e. It gives the mean concentration of the 3 50ppm & 100ppm treatment tanks by sampling location
 * (bottom/suspended) over the entire exposure
 *****************************************************************************************************
title "Mean treatment concentration by treatment group and sampling location for all sampling times";
proc sort;
by thero loc;

proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for each treatment group by sampling time
 * i.e. It gives the mean conc. of the 3 50ppm & 100ppm  treatment tanks at time 1, 3, 6, 9 and 12h
 *****************************************************************************************************
title "Mean treatment concentration for each treatment group for each sampling time";

proc sort;
by thero time loc;


proc means data = zec2 mean std lclm uclm fw=8;
by thero time loc;
var predicted_ppm;
run;

data zeq3; set output_out;
if conc > 1 then delete;
if tank > 0.5 then delete;
if thero = "." then delete;
run;

proc sort;
by thero;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for the standard checks for all time periods
 * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at 9 and 12h
 *****************************************************************************************************
title "Mean concentration for standard checks for all sampling times";
proc means data = zeq3 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
run;
quit;
run;
```

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```
DM 'LOG; CLEAR; OUTPUT; CLEAR;'; * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Lake Carlos-injection treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  30Jan2014  Analysis prepared by: JAL';

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  JAL        Page ___ of ___        *
* Analysis completion date:  30Jan2014  Jn                                    *
*****************************************************************************/

data Zeq; set carlos.carlosinjection;
run;

NOTE: There were 73 observations read from the data set CARLOS.CARLOSINJECTION.
NOTE: The data set WORK.ZEQ has 73 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by tank time loc; run;

NOTE: There were 73 observations read from the data set WORK.ZEQ.
NOTE: The data set WORK.ZEQ has 73 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


run;
proc gplot data= zeq;
plot abs * conc;
run;

NOTE: 61 observation(s) contained a MISSING value for the abs * conc request.
NOTE: 4 records written to C:\Users\JLUOMA\gplot1.png.

NOTE: There were 73 observations read from the data set WORK.ZEQ.
NOTE: PROCEDURE GPLOT used (Total process time):
      real time           0.26 seconds
      cpu time            0.25 seconds


proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
```

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```
NOTE: The data set WORK.OUTPUT_OUT has 73 observations and 7 variables.
NOTE: PROCEDURE REG used (Total process time):
      real time           1.04 seconds
      cpu time            0.42 seconds
```

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```
138  proc sort;
139  by time tank loc;

NOTE: There were 73 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.OUTPUT_OUT has 73 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


140  proc print data=output_out;
141  run;

NOTE: There were 73 observations read from the data set WORK.OUTPUT_OUT.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.04 seconds
      cpu time            0.04 seconds


142  data zeq2; set output_out;
143  if tank = "." then delete;
144  if loc =".* then delete;
145  if time = "0" then delete;
146  run;

NOTE: Character values have been converted to numeric values at the places given by:
      (Line):(Column).
      145:11
NOTE: There were 73 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ2 has 49 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


147  proc sort;
148  by tank loc;
149  run;

NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 49 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds
```



```
150  /*************************************************************************************************
150! **************
```

```
  * This procedure produces the mean concentrations for each treatment replicate over all
sampling times  *
  * by the the sampling location
               *
  * i.e. It gives the mean concentration of each treatment tank over the entire exposure by
the sampling *
  * location [i.e. bottom vs suspended sampling (bottom sampling initiated at 6h)]
               *
  *****************************************************************************************
**************/
title "Mean treatment concentration by treatment tank and sampling location
(bottom/suspended) for all sampling times";
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;

NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.04 seconds
      cpu time            0.03 seconds


proc sort;
by thero time loc;
/****************************************************************************************
**************
  * This procedure produces the mean concentrations for each treatment group over all
sampling times      *
  * by the sampling location
               *
  * i.e. It gives the mean concentration of the 3 50ppm & 100ppm treatment tanks by sampling
location     *
  * (bottom/suspended) over the entire exposure
               *
  *****************************************************************************************
**************/
title "Mean treatment concentration by treatment group and sampling location for all
sampling times";

NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 49 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by thero loc;


NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 49 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

```
173  proc means data = zeq2 mean std lclm uclm fw=8;
174  by thero loc;
175  var predicted_ppm;
176  run;
```

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```
NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.04 seconds
      cpu time            0.00 seconds


177  /*****************************************************************************************
177! **************
178   * This procedure produces the mean concentrations for each treatment group by sampling time
178!          *
179   * i.e. It gives the mean conc. of the 3 50ppm & 100ppm  treatment tanks at time 1, 3, 6, 9
179! and 12h      *
180   *****************************************************************************************
180! **************/
181  title 'Mean treatment concentration for each treatment group for each sampling time";
182
183  proc sort;
184  by thero time loc;
185
186

NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 49 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.00 seconds


187  proc means data = zeq2 mean std lclm uclm fw=8;
188  by thero time loc;
189  var predicted_ppm;
190  run;

NOTE: There were 49 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.07 seconds
      cpu time            0.04 seconds


191
192  data zeq3; set output_out;
193  if conc > 1 then delete;
194  if tank > 0.5 then delete;
195  if thero = "." then delete;
196  run;
```

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```
NOTE: Character values have been converted to numeric values at the places given by:
      (Line):(Column).
      194:4    195:12
```

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```
NOTE: There were 73 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ3 has 12 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc sort;
by thero;
/*************************************************************************************************
**************
 * This procedure produces the mean concentrations for the standard checks for all time
periods          *
 * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at 9 and 12h
            *
*************************************************************************************************
**************/
title "Mean concentration for standard checks for all sampling times";

NOTE: There were 12 observations read from the data set WORK.ZEQ3.
NOTE: The data set WORK.ZEQ3 has 12 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc means data = zeq3 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
run;

NOTE: There were 12 observations read from the data set WORK.ZEQ3.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.04 seconds
      cpu time            0.04 seconds


quit;
run;
```

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JA

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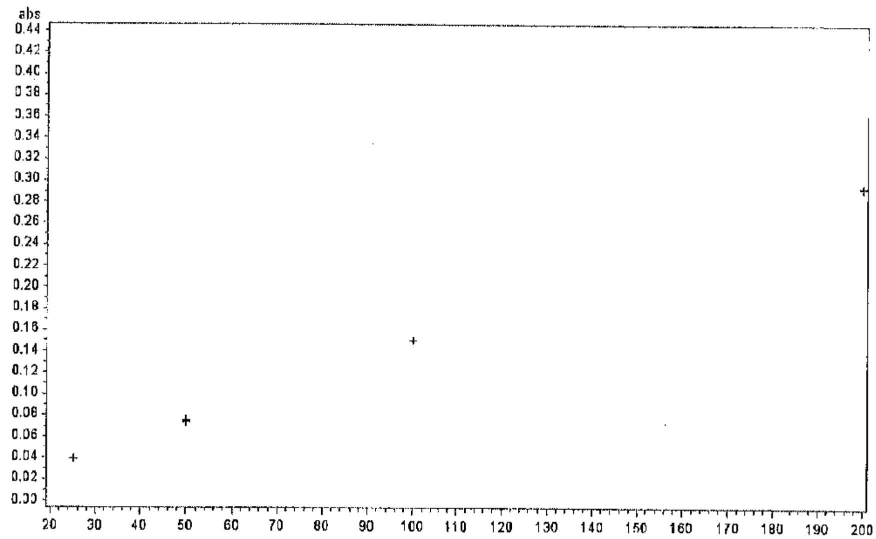
Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-Injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL

AEH-12-PSEUDO-04




Performed by J. Luoma SAS version 9.3 08.07 30JAN14

File Folder: 12c

Item Number: 4

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Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc

| | |
|---|---|
| Number of Observations Read | 73 |
| Number of Observations Used | 12 |
| Number of Observations with Missing Values | 61 |

| Analysis of Variance | | | | | |
|---|---|---|---|---|---|
| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
| Model | 1 | 53901 | 53901 | 95117.5 | <.0001 |
| Error | 10 | 5.66674 | 0.56667 | | |
| Corrected Total | 11 | 53906 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 0.75278 | R-Square | 0.9999 |
| Dependent Mean | 93.75000 | Adj R-Sq | 0.9999 |
| Coeff Var | 0.80296 | | |

| Parameter Estimates | | | | | | |
|---|---|---|---|---|---|---|
| Variable | Label | DF | Parameter Estimate | Standard Error | t Value | Pr > \|t\| |
| Intercept | Intercept | 1 | -1.77327 | 0.37836 | -4.69 | 0.0009 |
| abs | abs | 1 | 683.52963 | 2.21629 | 308.41 | <.0001 |

Performed by J. Luoma SAS version 9.3 08:07 30JAN14

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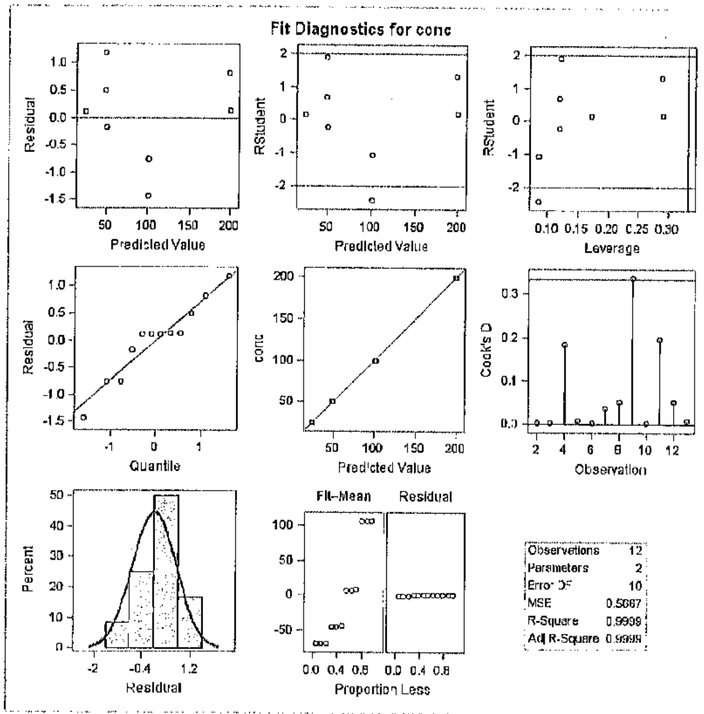
Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Lake Carlos-injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL

**The REG Procedure**
**Model: MODEL1**
**Dependent Variable: conc conc**



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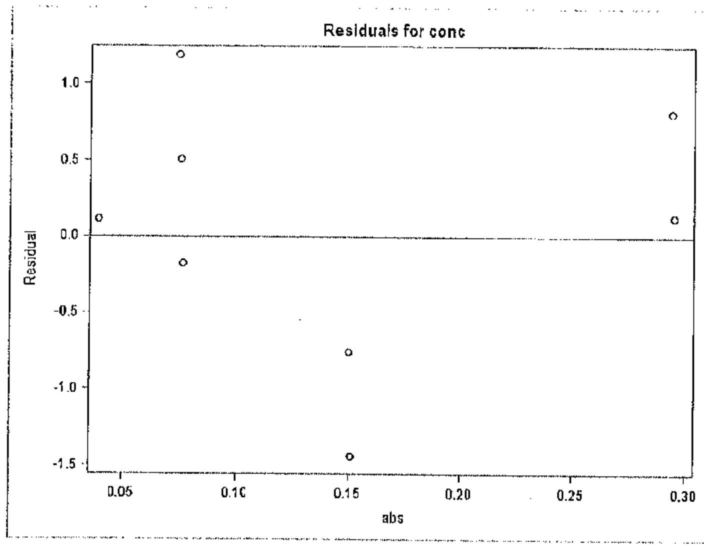


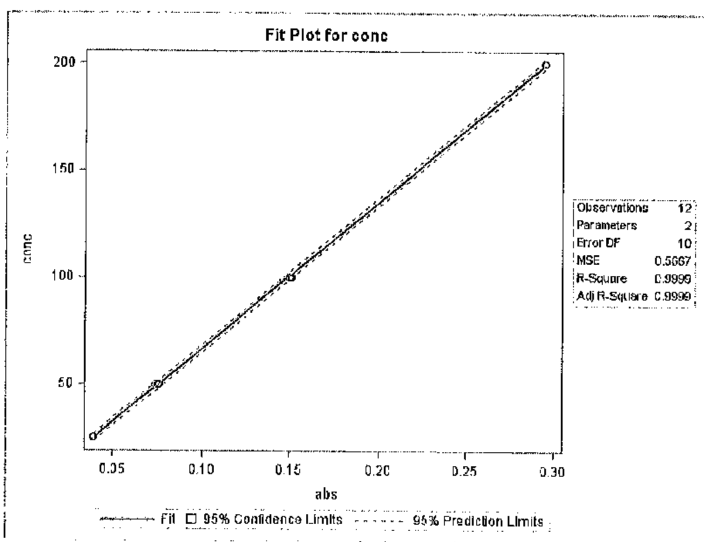


Performed by J. Luoma SAS version 9.3 08:07 30JAN14

**Standard Curve Linear Regression and sample concentrations**
**Study # AEH-12-PSUEDO-04**
**Lake Carlos-Injection treatment**
**SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL**

| Obs | tank | thero | time | loc | abs | conc | predicted_ppm |
|---|---|---|---|---|---|---|---|
| 1 | | . | . | | . | . | . |
| 2 | . | 25 | 0 | . | 0.039 | 25 | 24.884 |
| 3 | . | 50 | 0 | . | 0.076 | 50 | 50.175 |
| 4 | . | 100 | 0 | . | 0.151 | 100 | 101.440 |
| 5 | . | 200 | 0 | . | 0.295 | 200 | 199.868 |
| 6 | . | 25 | 0 | . | 0.039 | 25 | 24.884 |
| 7 | . | 50 | 0 | . | 0.075 | 50 | 49.491 |
| 8 | . | 100 | 0 | . | 0.150 | 100 | 100.756 |
| 9 | . | 200 | 0 | . | 0.294 | 200 | 199.184 |
| 10 | . | 25 | 0 | . | 0.039 | 25 | 24.884 |
| 11 | . | 50 | 0 | . | 0.074 | 50 | 48.808 |
| 12 | . | 100 | 0 | . | 0.150 | 100 | 100.756 |
| 13 | . | 200 | 0 | . | 0.295 | 200 | 199.868 |
| 14 | 1 | 50 | 1 | sus | 0.000 | . | -1.773 |
| 15 | 2 | 100 | 1 | sus | 0.009 | . | 4.379 |
| 16 | 4 | 50 | 1 | sus | 0.002 | . | -0.406 |
| 17 | 5 | 100 | 1 | sus | 0.003 | . | 0.277 |
| 18 | 8 | 50 | 1 | sus | 0.001 | . | -1.090 |
| 19 | 9 | 100 | 1 | sus | 0.036 | . | 22.834 |
| 20 | 1 | 50 | 3 | sus | 0.001 | . | -1.090 |
| 21 | 2 | 100 | 3 | sus | 0.001 | . | -1.090 |
| 22 | 4 | 50 | 3 | sus | 0.001 | . | -1.090 |
| 23 | 5 | 100 | 3 | sus | 0.002 | . | -0.406 |
| 24 | 8 | 50 | 3 | sus | 0.000 | . | -1.773 |
| 25 | 9 | 100 | 3 | sus | 0.000 | . | -1.773 |
| 26 | . | 25 | 6 | . | 0.036 | . | 22.834 |
| 27 | . | 50 | 6 | . | 0.065 | . | 42.656 |
| 28 | . | 100 | 6 | . | 0.135 | . | 90.503 |
| 29 | . | 200 | 6 | . | 0.270 | . | 182.780 |
| 30 | 1 | 50 | 6 | bot | 0.171 | . | 115.110 |
| 31 | 1 | 50 | 6 | sus | 0.005 | . | 1.644 |
| 32 | 2 | 100 | 6 | bot | 0.430 | . | 292.144 |
| 33 | 2 | 100 | 6 | sus | 0.006 | . | 2.328 |
| 34 | 4 | 50 | 6 | bot | 0.187 | . | 126.047 |
| 35 | 4 | 50 | 6 | sus | 0.005 | . | 1.644 |
| 36 | 5 | 100 | 6 | bot | 0.398 | . | 270.272 |
| 37 | 5 | 100 | 6 | sus | 0.001 | . | -1.090 |
| 38 | 8 | 50 | 6 | bot | 0.207 | . | 139.717 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 39 | 8 | 50 | 6 | sus | 0.001 | . | -1.090 |
| 40 | 9 | 100 | 6 | bot | 0.345 | . | 234.044 |
| 41 | 9 | 100 | 6 | sus | 0.000 | . | -1.773 |
| 42 | . | 25 | 9 | . | 0.033 | . | 20.783 |
| 43 | . | 50 | 9 | . | 0.069 | . | 45.390 |
| 44 | . | 100 | 9 | . | 0.135 | . | 90.503 |
| 45 | . | 200 | 9 | . | 0.265 | . | 179.362 |
| 46 | 1 | 50 | 9 | bot | 0.159 | . | 106.908 |
| 47 | 1 | 50 | 9 | sus | 0.004 | . | 0.961 |
| 48 | 2 | 100 | 9 | bot | 0.395 | . | 268.221 |
| 49 | 2 | 100 | 9 | sus | 0.006 | . | 2.328 |
| 50 | 4 | 50 | 9 | bot | 0.154 | . | 103.490 |
| 51 | 4 | 50 | 9 | sus | 0.001 | . | -1.090 |
| 52 | 5 | 100 | 9 | bot | 0.357 | . | 242.247 |
| 53 | 5 | 100 | 9 | sus | 0.001 | . | -1.090 |
| 54 | 8 | 50 | 9 | bot | 0.131 | . | 87.769 |
| 55 | 8 | 50 | 9 | sus | 0.003 | . | 0.277 |
| 56 | 9 | 100 | 9 | bot | 0.322 | . | 218.323 |
| 57 | 9 | 100 | 9 | sus | 0.003 | . | 0.277 |
| 58 | . | 25 | 12 | . | 0.034 | . | 21.467 |
| 59 | . | 50 | 12 | . | 0.068 | . | 44.707 |
| 60 | . | 100 | 12 | . | 0.135 | . | 90.503 |
| 61 | . | 200 | 12 | . | 0.265 | . | 179.362 |
| 62 | 1 | 50 | 12 | bot | 0.108 | . | 72.048 |
| 63 | 1 | 50 | 12 | sus | 0.011 | . | 5.746 |
| 64 | 2 | 100 | 12 | bot | 0.324 | . | 2[illegible]9.690 |
| 65 | 2 | 100 | 12 | sus | 0.004 | . | 0.961 |
| 66 | 4 | 50 | 12 | bot | 0.[illegible]13 | . | 75.466 |
| 67 | 4 | 50 | 12 | sus | 0.012 | . | 6.429 |
| 68 | 5 | 100 | 12 | bot | 0.285 | . | 193.033 |
| 69 | 5 | 100 | 12 | sus | 0.005 | . | 1.644 |
| 70 | 8 | 50 | 12 | bot | 0.114 | . | 76.149 |
| 71 | 8 | 50 | 12 | sus | 0.013 | . | 7.113 |
| 72 | 9 | 100 | 12 | bot | 0.257 | . | 173.894 |
| 73 | 9 | 100 | 12 | sus | 0.012 | . | 6.429 |

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**Mean treatment concentration by treatment tank and sampling location (bottom/suspended) for all sampling times**

The MEANS Procedure

tank=' ' loc=' '

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| . | . | . | . |

tank=1 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 98.0221 | 22.8651 | 41.2221 | 154.8 |

tank=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 1.0976 | 2.9558 | -2.5726 | 4.7677 |

tank=2 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 260.0 | 36.9169 | 168.3 | 351.7 |

tank=2 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 1.7811 | 2.0161 | -0.7223 | 4.2844 |

tank=4 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 101.7 | 25.3398 | 38.7200 | 164.6 |

tank=4 loc=sus

| Analysis Variable : predicted_ppm Predicted |
|---|

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| Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 1.0976 | 3.1841 | -2.8560 | 5.0511 |

tank=5 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 235.2 | 39.1008 | 138.1 | 332.3 |

tank=5 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.1328 | 1.1438 | -1.5530 | 1.2874 |

tank=8 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 101.2 | 33.8491 | 17.1260 | 185.3 |

tank=8 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 0.6874 | 3.6682 | -3.8672 | 5.2421 |

tank=9 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 208.8 | 31.1962 | 131.3 | 286.2 |

tank=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 5.1987 | 10.4157 | -7.7341 | 18.1315 |

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**Mean treatment concentration by treatment group and sampling location for all sampling times**

**The MEANS Procedure**

thero=. loc=' '

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| . | . | . | . |

thero=50 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 100.3 | 24.0962 | 81.7785 | 118.8 |

thero=50 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 0.9609 | 3.0459 | -0.7259 | 2.6476 |

thero=100 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 234.7 | 38.1959 | 205.3 | 264.0 |

thero=100 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 2.2823 | 6.1434 | -1.1198 | 5.6845 |

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**Mean treatment concentration for each treatment group for each sampling time**

**The MEANS Procedure**

thero=. time=. loc=' '

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| . | . | . | . |

thero=50 time=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.0897 | 0.6835 | -2.7877 | 0.6082 |

thero=50 time=3 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.3176 | 0.3946 | -2.2979 | -0.3372 |

thero=50 time=6 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 127.0 | 12.3288 | 96.3317 | 157.6 |

thero=50 time=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 0.7330 | 1.5765 | -3.1883 | 4.6543 |

thero=50 time=9 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 99.3891 | 10.2073 | 74.0328 | 124.7 |

thero=50 time=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 0.0495 | 1.0441 | -2.5442 | 2.6432 |

thero=50 time=12 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 74.5542 | 2.1972 | 69.0960 | 80.0125 |

thero=50 time=12 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 6.4291 | 0.6835 | 4.7311 | 8.1271 |

thero=100 time=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 9.1632 | 12.0154 | -20.6846 | 39.0110 |

thero=100 time=3 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.0897 | 0.6835 | -2.7877 | 0.6082 |

thero=100 time=6 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 265.5 | 29.3440 | 192.6 | 338.4 |

thero=100 time=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.1784 | 2.1972 | -5.6366 | 5.2799 |

thero=100 time=9 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 242.9 | 24.9559 | 180.9 | 304.9 |

thero=100 time=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 0.5052 | 1.7202 | -3.7680 | 4.7783 |

thero=100 time=12 loc=bot

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 195.5 | 23.0009 | 138.4 | 252.7 |

thero=100 time=12 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 3.0114 | 2.9794 | -4.3899 | 10.4128 |

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**Mean concentration for standard checks for all sampling times**

The MEANS Procedure

thero=25

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 21.6946 | 1.0441 | 19.1009 | 24.2883 |

thero=50

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 44.2511 | 1.4229 | 40.7164 | 47.7857 |

thero=100

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 90.5032 | 0 | . | . |

thero=200

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 180.5 | 1.9732 | 175.6 | 185.4 |

Performed by J. Luoma SAS version 9.3 08:07 30JAN14 JL

File Folder: 12c Item Number: 4

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 25 - 27)
Data Source: File Folder: 14c
Forms: "Sample Absorbance Readings" Data Sheet

File Name: See filenames as stated below

| Action | Date | Initials |
|---|---|---|
| Created...... | 6-Feb-14 | TJS TJS |
| Revised...... | 12-Feb-14 | TJS TJS |
| Reviewed... | 12FEB14 | TJS |
| Certified...... | 2/19/14 | JAV |

**Spectrophotometric Data**

Test Article: Zequanox ® (MBI-401 SDP)
Test Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

**Data Explanation:**

1) The absorbance of triplicate samples of 25, 50, 100, and 200 mg/L dilutions of a 2,000 mg/L active ingredient (A.I.) stock prepared from Analytical Stock #2 were measured to prepare a standard curve.
2) Standard checks were performed at 6, 9, and 12 hours by comparing the 25, 50, 100, and 200 mg/L (A.I.) dilutions to the linear curve.
3) Data codes used within SAS
- tank = Tank ID (1 through 9)
- thero = Theoretical or target concentration (mg/L)
- time = Sample Time (0, 1, 3, 6, 9, and 12 h after treatment)
- loc = Sample Location
  - sus = Suspended Sample (sampled ~15 cm from bottom of tank)
  - sur = Surface Sample
- abs = measured absorbance of sample
- conc = concentration ([mg/L], only used for standards used for regression)

4) Information that is not relevant to a sample (i.e., tank ID for standards) or that will be calculated by SAS (i.e., predicted concentration for standard checks and samples) is denoted by a "." in the SAS input and output files.

**Data Analysis:**

1) A linear regression was completed in SAS using the absorbance values obtained from the spectrophotometer of 3 replicate dilutions of 25, 50, 100 and 200 mg/L
2) Standard checks and treatment sample concentrations were predicted in SAS by comparing the observed absorbances with the linear regression.
3) The following mean treatment concentrations were determined in SAS:
- 3a) Mean (standard deviation) concentration by tank and location for all sampling times
- 3b) Mean (standard deviation) concentration by treatment group and location for all sampling times
- 3c) Mean (standard deviation) concentration by treatment group and sampling times for both locations
- 3d) Mean (standard deviation) concentrations for 25, 50, 100, and 200 mg/L (A.I.) dilutions for all sampling times

**File Names:**

Spectrophotometric Data for SAS Input
I:\AEH-12-PSEUDO-04\Data Summaries\spec\[Lake Shawano Bottom Injection Spec Summary.xlsx]Spec Data for SAS

SAS Program/Code
I:\AEH-12-PSEUDO-04\SAS-Spec\shawano injection program file

SAS Log
I:\AEH-12-PSEUDO-04\SAS-Spec\shawano injection log file

SAS Output
I:\AEH-12-PSEUDO-04\SAS-Spec\Shawano injection results file

**Data Anomalies and Deviations:**

1) One exposure tank of a different concentration was sampled in triplicate to evaluate variability of spectrophotometer during each sampling time. The triplicate sample was only taken from suspended (sus) sampling locations. The mean absorbance of the triplicate samples was imported into SAS for use in the analysis.
2) Surface samples (sur) were collected by submerging a collection beaker below the surface of each exposure tank; suspended samples (sus) were collected ~15 cm from the exposure tank bottom for all sampling times.
3) Some mean absorbances for triplicate samples may be recorded incorrectly on "Sample Absorbance Readings" data forms as proper significant figure rules may not have been observed. Additionally, concentrations recorded on "Sample Absorbance Readings" data forms were not used in the analysis as the initial linear regression equation that was used for these calculations was derived using rounded absorbance values in Excel. All absorbances and concentrations used in SAS calculations and reported within Spectrophotometric Data Summary have been corrected.

Item Number 1
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File Folder: 14c

AEH-12-PSEUDO-04

| tank | thero | time | loc | abs | conc |
|---|---|---|---|---|---|
| . | 25 | 0 | . | 0.038 | 25 |
| . | 50 | 0 | . | 0.067 | 50 |
| . | 100 | 0 | . | 0.129 | 100 |
| . | 200 | 0 | . | 0.250 | 200 |
| . | 25 | 0 | . | 0.036 | 25 |
| . | 50 | 0 | . | 0.065 | 50 |
| . | 100 | 0 | . | 0.128 | 100 |
| . | 200 | 0 | . | 0.253 | 200 |
| . | 25 | 0 | . | 0.037 | 25 |
| . | 50 | 0 | . | 0.066 | 50 |
| . | 100 | 0 | . | 0.130 | 100 |
| . | 200 | 0 | . | 0.251 | 200 |
| . | 25 | 6 | . | 0.037 | . |
| . | 50 | 6 | . | 0.066 | . |
| . | 100 | 6 | . | 0.127 | . |
| . | 200 | 6 | . | 0.247 | . |
| . | 25 | 9 | . | 0.037 | . |
| . | 50 | 9 | . | 0.066 | . |
| . | 100 | 9 | . | 0.115 | . |
| . | 200 | 9 | . | 0.243 | . |
| . | 25 | 12 | . | 0.038 | . |
| . | 50 | 12 | . | 0.065 | . |
| . | 100 | 12 | . | 0.127 | . |
| . | 200 | 12 | . | 0.243 | . |
| 1 | 0 | 1 | sur | 0.002 | . |
| 8 | 0 | 1 | sur | 0.002 | . |
| 9 | 0 | 1 | sur | 0.003 | . |
| 2 | 50 | 1 | sur | 0.004 | . |
| 3 | 50 | 1 | sur | 0.001 | . |
| 7 | 50 | 1 | sur | 0.002 | . |
| 4 | 100 | 1 | sur | 0.003 | . |
| 5 | 100 | 1 | sur | 0.004 | . |
| 6 | 100 | 1 | sur | 0.004 | . |
| 1 | 0 | 1 | sus | 0.002 | . |
| 8 | 0 | 1 | sus | 0.001 | . |
| 9 | 0 | 1 | sus | 0.002 | . |
| 2 | 50 | 1 | sus | 0.075 | . |
| 3 | 50 | 1 | sus | 0.068 | . |
| 7 | 50 | 1 | sus | 0.067 | . |
| 4 | 100 | 1 | sus | 0.144 | . |
| 5 | 100 | 1 | sus | 0.133 | . |
| 6 | 100 | 1 | sus | 0.119 | . |
| 1 | 0 | 3 | sur | 0.002 | . |
| 8 | 0 | 3 | sur | 0.002 | . |
| 9 | 0 | 3 | sur | 0.002 | . |
| 2 | 50 | 3 | sur | 0.004 | . |

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| | | | | | |
|---|---|---|---|---|---|
| 3 | 50 | 3 | sur | 0.002 | . |
| 7 | 50 | 3 | sur | 0.004 | . |
| 4 | 100 | 3 | sur | 0.002 | . |
| 5 | 100 | 3 | sur | 0.000 | . |
| 6 | 100 | 3 | sur | 0.003 | . |
| 1 | 0 | 3 | sus | 0.002 | . |
| 8 | 0 | 3 | sus | 0.003 | . |
| 9 | 0 | 3 | sus | 0.003 | . |
| 2 | 50 | 3 | sus | 0.055 | . |
| 3 | 50 | 3 | sus | 0.055 | . |
| 7 | 50 | 3 | sus | 0.054 | . |
| 4 | 100 | 3 | sus | 0.124 | . |
| 5 | 100 | 3 | sus | 0.126 | . |
| 6 | 100 | 3 | sus | 0.114 | . |
| 1 | 0 | 6 | sur | 0.000 | . |
| 8 | 0 | 6 | sur | 0.000 | . |
| 9 | 0 | 6 | sur | 0.000 | . |
| 2 | 50 | 6 | sur | 0.009 | . |
| 3 | 50 | 6 | sur | 0.006 | . |
| 7 | 50 | 6 | sur | 0.007 | . |
| 4 | 100 | 6 | sur | 0.003 | . |
| 5 | 100 | 6 | sur | 0.003 | . |
| 6 | 100 | 6 | sur | 0.003 | . |
| 1 | 0 | 6 | sus | 0.000 | . |
| 8 | 0 | 6 | sus | 0.000 | . |
| 9 | 0 | 6 | sus | 0.001 | . |
| 2 | 50 | 6 | sus | 0.051 | . |
| 3 | 50 | 6 | sus | 0.048 | . |
| 7 | 50 | 6 | sus | 0.047 | . |
| 4 | 100 | 6 | sus | 0.119 | . |
| 5 | 100 | 6 | sus | 0.115 | . |
| 6 | 100 | 6 | sus | 0.106 | . |
| 1 | 0 | 9 | sur | 0.004 | . |
| 8 | 0 | 9 | sur | 0.003 | . |
| 9 | 0 | 9 | sur | 0.003 | . |
| 2 | 50 | 9 | sur | 0.013 | . |
| 3 | 50 | 9 | sur | 0.010 | . |
| 7 | 50 | 9 | sur | 0.018 | . |
| 4 | 100 | 9 | sur | 0.009 | . |
| 5 | 100 | 9 | sur | 0.010 | . |
| 6 | 100 | 9 | sur | 0.007 | . |
| 1 | 0 | 9 | sus | 0.003 | . |
| 8 | 0 | 9 | sus | 0.004 | . |
| 9 | 0 | 9 | sus | 0.002 | . |
| 2 | 50 | 9 | sus | 0.053 | . |
| 3 | 50 | 9 | sus | 0.054 | . |
| 7 | 50 | 9 | sus | 0.048 | . |

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| | | | | | |
|---|---|---|---|---|---|
| 4 | 100 | 9 | sus | 0.127 | . |
| 5 | 100 | 9 | sus | 0.119 | . |
| 6 | 100 | 9 | sus | 0.114 | . |
| 1 | 0 | 12 | sur | 0.004 | . |
| 8 | 0 | 12 | sur | 0.004 | . |
| 9 | 0 | 12 | sur | 0.004 | . |
| 2 | 50 | 12 | sur | 0.016 | . |
| 3 | 50 | 12 | sur | 0.015 | . |
| 7 | 50 | 12 | sur | 0.028 | . |
| 4 | 100 | 12 | sur | 0.010 | . |
| 5 | 100 | 12 | sur | 0.011 | . |
| 6 | 100 | 12 | sur | 0.009 | . |
| 1 | 0 | 12 | sus | 0.003 | . |
| 8 | 0 | 12 | sus | 0.004 | . |
| 9 | 0 | 12 | sus | 0.004 | . |
| 2 | 50 | 12 | sus | 0.053 | . |
| 3 | 50 | 12 | sus | 0.046 | . |
| 7 | 50 | 12 | sus | 0.025 | . |
| 4 | 100 | 12 | sus | 0.115 | . |
| 5 | 100 | 12 | sus | 0.113 | . |
| 6 | 100 | 12 | sus | 0.109 | . |

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```
ods html close; /* close previous */;
ods html; /* open new */;
ods graphics on;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;
```

AEH-12-PSEUDO-04

```
FOOTNOTE1 'Performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Shawano Lake-injection treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  30Jan2014  Analysis prepared by: JAL';

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  JAL        Page ___ of ___        *
* Analysis completion date:  30Jan2014  SN                                   *
*****************************************************************************/

data Zeq; set shawano.shawanoinjection;
run;
proc sort;
by tank time loc; run;

run;
proc gplot data= zeq;
plot abs * conc;
run;
proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
proc sort;
by time tank loc;
proc print data=output_out;
run;
data zeq2; set output_out;
if tank = "." then delete;
if loc ="." then delete;
if time = "0" then delete;
run;
proc sort;
by tank loc;
run;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for each treatment replicate over all sampling tim
 * by the the sampling location
 * i.e. It gives the mean concentration of each treatment tank over the entire exposure by  the sampl
 * location [i.e. surface vs suspended sampling
 ****************************************************************************************************
title "Mean treatment concentration by treatment tank and sampling location (surface/suspended) for a
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;
proc sort;
```

File Folder: 14C  Item Number: 2  Page 1 of 2

```
by thero time loc;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for each treatment group over all sampling times
 * by the sampling location
 * i.e. It gives the mean concentration of the 3 control, 50ppm & 100ppm treatment tanks by
 * sampling location (surface/suspended) over the entire exposure
 ****************************************************************************************************
title "Mean treatment concentration by treatment group and sampling location for all sampling times";
proc sort;
by thero loc;

proc means data = zeq2 mean std lclm uclm fw=8;
by thero loc;
var predicted_ppm;
run;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for each treatment group by sampling time
 * i.e. It gives the mean conc. of the 3 control,50ppm & 100ppm  treatment tanks at time 1, 3, 6, 9 a
 ****************************************************************************************************
title "Mean treatment concentration for each treatment group for each sampling time";

proc sort;
by thero time loc;


proc means data = zeq2 mean std lclm uclm fw=8;
by thero time loc;
var predicted_ppm;
run;

data zeq3; set output_out;
if conc > 1 then delete;
if tank > 0.5 then delete;
if thero = "." then delete;
run;

proc sort;
by thero;
/*****************************************************************************************************
 * This procedure produces the mean concentrations for the standard checks for all time periods
 * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at 6, 9 and 12h
 ****************************************************************************************************
title "Mean concentration for standard checks for all sampling times";
proc means data = zeq3 mean std lclm uclm fw=8;
by thero;
var predicted_ppm;
run;
quit;
run;
```

1/3/14
JRC

File Folder: 14C    Item Number: 2    Page 2 of 2

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by J. Luoma SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or unquoted text.

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Standard Curve Linear Regression and sample concentrations';
title2 h=1 'Study # AEH-12-PSUEDO-04';
title3 h=1 'Shawano Lake-injection treatment';
title4 h=1 'SAS v. 9.3  Analysis completion date:  30Jan2014  Analysis prepared by: JAL';

/*****************************************************************************
* SAS ver 9.3       Analysis prepared by:  JAL        Page ___ of ___          *
* Analysis completion date:  30Jan2014  JAL                                    *
*****************************************************************************/

data Zeq; set shawano.shawanoinjection;
run;

NOTE: There were 115 observations read from the data set SHAWANO.SHAWANOINJECTION.
NOTE: The data set WORK.ZEQ has 115 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


proc sort;
by tank time loc; run;

NOTE: There were 115 observations read from the data set WORK.ZEQ.
NOTE: The data set WORK.ZEQ has 115 observations and 6 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds



run;
proc gplot data= zeq;
plot abs * conc;
run;

NOTE: 103 observation(s) contained a MISSING value for the abs * conc request.
NOTE: 4 records written to C:\Users\JLUOMA\gplot4.png.

NOTE: There were 115 observations read from the data set WORK.ZEQ.
NOTE: PROCEDURE GPLOT used (Total process time):
      real time           0.26 seconds
      cpu time            0.26 seconds

proc reg data = zeq;
model conc = abs /edf;
output out=output_out p=predicted_ppm;
run;
```

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```
NOTE: The data set WORK.OUTPUT_OUT has 115 observations and 7 variables.
NOTE: PROCEDURE REG used (Total process time):
      real time           1.06 seconds
      cpu time            0.40 seconds


455  proc sort;
456  by time tank loc;

NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.OUTPUT_OUT has 115 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds


457  proc print data=output_out;
458  run;

NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.06 seconds
      cpu time            0.06 seconds


459  data zeq2; set output_out;
460  if tank = "." then delete;
461  if loc =" ." then delete;
462  if time = "0" then delete;
463  run;

NOTE: Character values have been converted to numeric values at the places given by:
      (Line):(Column).
      462:11
NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ2 has 91 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


464  proc sort;
465  by tank loc;
466  run;

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 91 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```



```
467  /*********************************************************************************************
467! **************
```

```
 * This procedure produces the mean concentrations for each treatment replicate over all
sampling times *
 * by the the sampling location
            *
 * i.e. It gives the mean concentration of each treatment tank over the entire exposure by
the sampling *
 * location [i.e. surface vs suspended sampling
            *
 *****************************************************************************************
**************/
title "Mean treatment concentration by treatment tank and sampling location
(surface/suspended) for all sampling tines";
proc means data = zeq2 mean std lclm uclm fw=8;
by tank loc;
var predicted_ppm;
run;

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.05 seconds
      cpu time            0.06 seconds


proc sort;
by thero time loc;
/****************************************************************************************
**************
 * This procedure produces the mean concentrations for each treatment group over all
sampling times       *
 * by the sampling location
            *
 * i.e. It gives the mean concentration of the 3 control, 50ppm & 100ppm treatment tanks by
            *
 * sampling location (surface/suspended) over the entire exposure
            *
 *****************************************************************************************
**************/
title "Mean treatment concentration by treatment group and sampling location for all
sampling times";

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 91 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.0´ seconds


proc sort;
by thero loc;

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 91 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.00 seconds
      cpu time            0.01 seconds
```

```
490  proc means data = zeq2 mean std lclm uclm fw=8;
491  by thero loc;
492  var predicted_ppm;
493  run;

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.04 seconds
      cpu time            0.01 seconds


494  /*****************************************************************************************
494! **********************
495   * This procedure produces the mean concentrations for each treatment group by sampling time
495!                       *
496   * i.e. It gives the mean conc. of the 3 control,50ppm & 100ppm  treatment tanks at time 1,
496! 3, 6, 9 and 12h       *
497   *****************************************************************************************
497! **********************/
498  title "Mean treatment concentration for each treatment group for each sampling time";
499
500  proc sort;
501  by thero time loc;
502
503

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: The data set WORK.ZEQ2 has 91 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


504  proc means data = zeq2 mean std lclm uclm fw=8;
505  by thero time loc;
506  var predicted_ppm;
507  run;

NOTE: There were 91 observations read from the data set WORK.ZEQ2.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.07 seconds
      cpu time            0.06 seconds


508
509  data zeq3; set output out;
510  if conc > 1 then delete;
511  if tank > 0.5 then delete;
512  if thero = "." then delete;
513  run;

NOTE: Character values have been converted to numeric values at the places given by:
      (Line):(Column).
      511:4    512:12
```

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```
NOTE: There were 115 observations read from the data set WORK.OUTPUT_OUT.
NOTE: The data set WORK.ZEQ3 has 12 observations and 7 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


514
515  proc sort;
516  by thero;
517  /******************************************************************************************
517! *************
518   * This procedure produces the mean concentrations for the standard checks for all time
518! periods         *
519   * i.e. It gives the mean conc. of the 50ppm & 100ppm standard checks at 6, 9 and 12h
519!            *
520  ******************************************************************************************
520! *************/
521  title "Mean concentration for standard checks for all sampling times";

NOTE: There were 12 observations read from the data set WORK.ZEQ3.
NOTE: The data set WORK.ZEQ3 has 12 observations and 7 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


522  proc means data = zeq3 mean std lclm uclm fw=8;
523  by thero;
524  var predicted_ppm;
525  run;

NOTE: There were 12 observations read from the data set WORK.ZEQ3.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.04 seconds
      cpu time            0.03 seconds


526  quit;
527  run;
```

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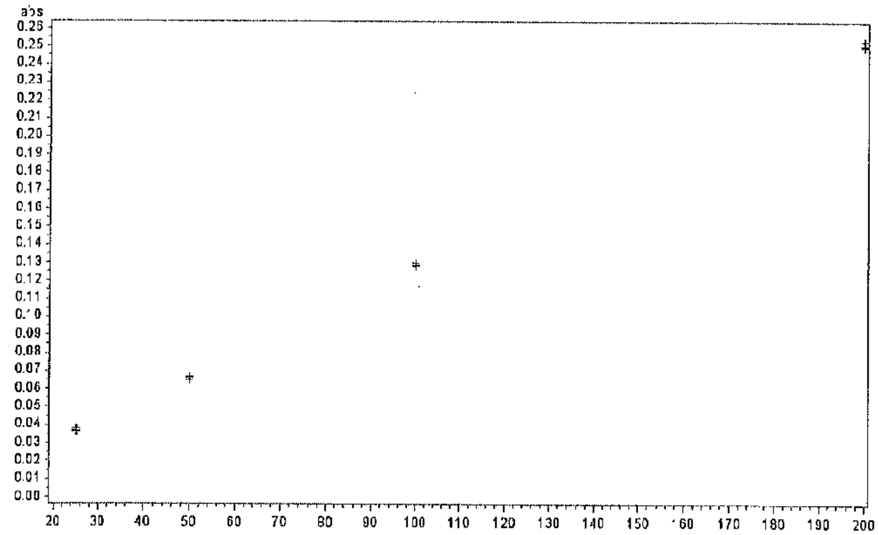
AEH-12-PSEUDO-04

Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-Injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL




Performed by J. Luoma SAS version 9.3 08:37 30JAN14

File Folder: 14c Item Number: 4

Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-Injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc

| | |
|---|---|
| Number of Observations Read | 115 |
| Number of Observations Used | 12 |
| Number of Observations with Missing Values | 103 |

Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 53896 | 53896 | 50557.1 | <.0001 |
| Error | 10 | 10.66035 | 1.06603 | | |
| Corrected Total | 11 | 53906 | | | |

| | | | |
|---|---|---|---|
| Root MSE | 1.03249 | R-Square | 0.9998 |
| Dependent Mean | 93.75000 | Adj R-Sq | 0.9998 |
| Coeff Var | 1.10132 | | |

Paramoter Estimates

| Variable | Label | DF | Parameter Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| Intercept | Intercept | 1 | -4.56868 | 0.52919 | -8.63 | <.0001 |
| abs | abs | 1 | 813.67186 | 3.61875 | 224.85 | <.0001 |

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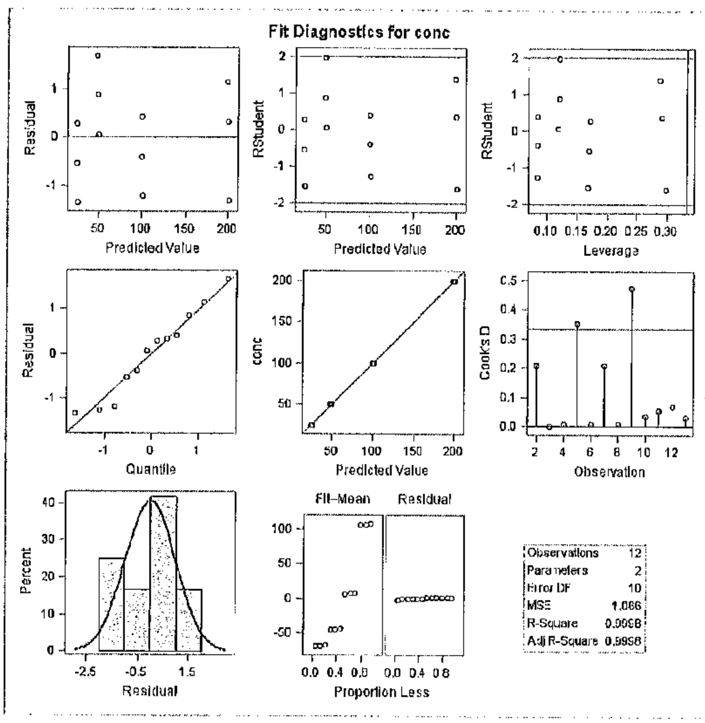
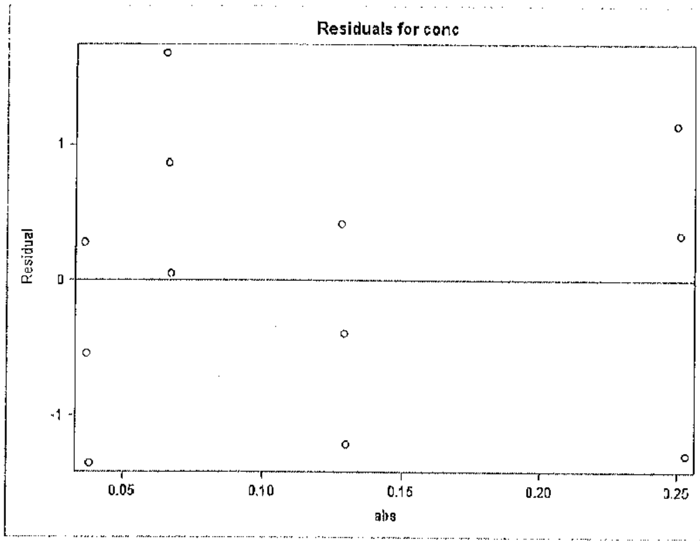
AEH-12-PSEUDO-04

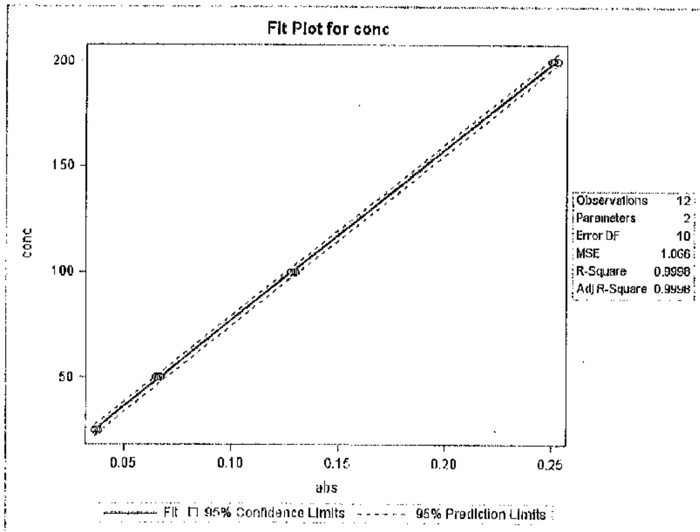
Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-Injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL

The REG Procedure
Model: MODEL1
Dependent Variable: conc conc



AEH-12-PSEUDO-04




Performed by J. Luoma SAS version 9.3 08:07 30JAN14

AEH-12-PSEUDO-04

Standard Curve Linear Regression and sample concentrations
Study # AEH-12-PSUEDO-04
Shawano Lake-Injection treatment
SAS v. 9.3 Analysis completion date: 30Jan2014 Analysis prepared by: JAL

| Obs | tank | thero | time | loc | abs | conc | predicted_ppm |
|---|---|---|---|---|---|---|---|
| 1 | | . | . | | . | . | . |
| 2 | . | 25 | 0 | . | 0.03800 | 25 | 26.351 |
| 3 | . | 50 | 0 | . | 0.06700 | 50 | 49.947 |
| 4 | . | 100 | 0 | . | 0.12900 | 100 | 100.395 |
| 5 | . | 200 | 0 | . | 0.25000 | 200 | 198.849 |
| 6 | . | 25 | 0 | . | 0.03600 | 25 | 24.724 |
| 7 | . | 50 | 0 | . | 0.06500 | 50 | 48.320 |
| 8 | . | 100 | 0 | . | 0.12800 | 100 | 99.581 |
| 9 | . | 200 | 0 | . | 0.25300 | 200 | 201.290 |
| 10 | . | 25 | 0 | . | 0.03700 | 25 | 25.537 |
| 11 | . | 50 | 0 | . | 0.06600 | 50 | 49.134 |
| 12 | . | 100 | 0 | . | 0.13000 | 100 | 101.209 |
| 13 | . | 200 | 0 | . | 0.25100 | 200 | 199.663 |
| 14 | 1 | 0 | 1 | sur | 0.00200 | . | -2.941 |
| 15 | 1 | 0 | 1 | sus | 0.00200 | . | -2.941 |
| 16 | 2 | 50 | 1 | sur | 0.00400 | . | -1.314 |
| 17 | 2 | 50 | 1 | sus | 0.07500 | . | 56.457 |
| 18 | 3 | 50 | 1 | sur | 0.00100 | . | -3.755 |
| 19 | 3 | 50 | 1 | sus | 0.06800 | . | 50.761 |
| 20 | 4 | 100 | 1 | sur | 0.00300 | . | -2.128 |
| 21 | 4 | 100 | 1 | sus | 0.14400 | . | 112.600 |
| 22 | 5 | 100 | 1 | sur | 0.00400 | . | -1.314 |
| 23 | 5 | 100 | 1 | sus | 0.13300 | . | 103.650 |
| 24 | 6 | 100 | 1 | sur | 0.00400 | . | -1.314 |
| 25 | 6 | 100 | 1 | sus | 0.11900 | . | 92.258 |
| 26 | 7 | 50 | 1 | sur | 0.00200 | . | -2.941 |
| 27 | 7 | 50 | 1 | sus | 0.06700 | . | 49.947 |
| 28 | 8 | 0 | 1 | sur | 0.00200 | . | -2.941 |
| 29 | 8 | 0 | 1 | sus | 0.00100 | . | -3.755 |
| 30 | 9 | 0 | 1 | sur | 0.00300 | . | -2.128 |
| 31 | 9 | 0 | 1 | sus | 0.00200 | . | -2.941 |
| 32 | 1 | 0 | 3 | sur | 0.00200 | . | -2.941 |
| 33 | 1 | 0 | 3 | sus | 0.00200 | . | -2.941 |
| 34 | 2 | 50 | 3 | sur | 0.00400 | . | -1.314 |
| 35 | 2 | 50 | 3 | sus | 0.05500 | . | 40.183 |
| 36 | 3 | 50 | 3 | sur | 0.00200 | . | -2.941 |
| 37 | 3 | 50 | 3 | sus | 0.05467 | . | 39.912 |
| 38 | 4 | 100 | 3 | sur | 0.00200 | . | -2.941 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 39 | 4 | 100 | 3 | sus | 0.12400 | . | 96.327 |
| 40 | 5 | 100 | 3 | sur | 0.00000 | . | -4.569 |
| 41 | 5 | 100 | 3 | sus | 0.12600 | . | 97.954 |
| 42 | 6 | 100 | 3 | sur | 0.00300 | . | -2.128 |
| 43 | 6 | 100 | 3 | sus | 0.11400 | . | 88.190 |
| 44 | 7 | 50 | 3 | sur | 0.00400 | . | -1.314 |
| 45 | 7 | 50 | 3 | sus | 0.05400 | . | 39.370 |
| 46 | 8 | 0 | 3 | sur | 0.00200 | . | -2.941 |
| 47 | 8 | 0 | 3 | sus | 0.00300 | . | -2.128 |
| 48 | 9 | 0 | 3 | sur | 0.00200 | . | -2.941 |
| 49 | 9 | 0 | 3 | sus | 0.00300 | . | -2.128 |
| 50 | . | 25 | 6 | . | 0.03700 | . | 25.537 |
| 51 | . | 50 | 6 | . | 0.06600 | . | 49.134 |
| 52 | . | 100 | 6 | . | 0.12700 | . | 98.768 |
| 53 | . | 200 | 6 | . | 0.24700 | . | 196.408 |
| 54 | 1 | 0 | 6 | sur | 0.00000 | . | -4.569 |
| 55 | 1 | 0 | 6 | sus | 0.00000 | . | -4.569 |
| 56 | 2 | 50 | 6 | sur | 0.00900 | . | 2.754 |
| 57 | 2 | 50 | 6 | sus | 0.05100 | . | 36.929 |
| 58 | 3 | 50 | 6 | sur | 0.00600 | . | 0.313 |
| 59 | 3 | 50 | 6 | sus | 0.04800 | . | 34.488 |
| 60 | 4 | 100 | 6 | sur | 0.00300 | . | -2.128 |
| 61 | 4 | 100 | 6 | sus | 0.11900 | . | 92.258 |
| 62 | 5 | 100 | 6 | sur | 0.00300 | . | -2.128 |
| 63 | 5 | 100 | 6 | sus | 0.11500 | . | 89.004 |
| 64 | 6 | 100 | 6 | sur | 0.00300 | . | -2.128 |
| 65 | 6 | 100 | 6 | sus | 0.10600 | . | 81.681 |
| 66 | 7 | 50 | 6 | sur | 0.00700 | . | 1.127 |
| 67 | 7 | 50 | 6 | sus | 0.04733 | . | 33.945 |
| 68 | 8 | 0 | 6 | sur | 0.00000 | . | -4.569 |
| 69 | 8 | 0 | 6 | sus | 0.00000 | . | -4.569 |
| 70 | 9 | 0 | 6 | sur | 0.00000 | . | -4.569 |
| 71 | 9 | 0 | 6 | sus | 0.00100 | . | -3.755 |
| 72 | . | 25 | 9 | . | 0.03700 | . | 25.537 |
| 73 | . | 50 | 9 | . | 0.06600 | . | 49.134 |
| 74 | . | 100 | 9 | . | 0.11500 | . | 89.004 |
| 75 | . | 200 | 9 | . | 0.24300 | . | 193.154 |
| 76 | 1 | 0 | 9 | sur | 0.00400 | . | -1.314 |
| 77 | 1 | 0 | 9 | sus | 0.00300 | . | -2.128 |
| 78 | 2 | 50 | 9 | sur | 0.01300 | . | 6.009 |
| 79 | 2 | 50 | 9 | sus | 0.05300 | . | 38.556 |
| 80 | 3 | 50 | 9 | sur | 0.01000 | . | 3.568 |
| 81 | | | | | | | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 50 | 9 | sus | 0.05400 | . | 39.370 |
| 82 | 4 | 100 | 9 | sur | 0.00900 | . | 2.754 |
| 83 | 4 | 100 | 9 | sus | 0.12733 | . | 99.039 |
| 84 | 5 | 100 | 9 | sur | 0.01000 | . | 3.568 |
| 85 | 5 | 100 | 9 | sus | 0.11900 | . | 92.258 |
| 86 | 6 | 100 | 9 | sur | 0.00700 | . | 1.127 |
| 87 | 6 | 100 | 9 | sus | 0.11400 | . | 88.190 |
| 88 | 7 | 50 | 9 | sur | 0.01600 | . | 10.077 |
| 89 | 7 | 50 | 9 | sus | 0.04800 | . | 34.488 |
| 90 | 8 | 0 | 9 | sur | 0.00300 | . | -2.128 |
| 91 | 8 | 0 | 9 | sus | 0.00400 | . | -1.314 |
| 92 | 9 | 0 | 9 | sur | 0.00300 | . | -2.128 |
| 93 | 9 | 0 | 9 | sus | 0.00200 | . | -2.941 |
| 94 | . | 25 | 12 | . | 0.03800 | . | 26.351 |
| 95 | . | 50 | 12 | . | 0.06500 | . | 48.320 |
| 96 | . | 100 | 12 | . | 0.12700 | . | 98.768 |
| 97 | . | 200 | 12 | . | 0.24300 | . | 193.154 |
| 98 | 1 | 0 | 12 | sur | 0.00400 | . | -1.314 |
| 99 | 1 | 0 | 12 | sus | 0.00300 | . | -2.128 |
| 100 | 2 | 50 | 12 | sur | 0.01600 | . | 8.460 |
| 101 | 2 | 50 | 12 | sus | 0.05300 | . | 38.556 |
| 102 | 3 | 50 | 12 | sur | 0.01500 | . | 7.636 |
| 103 | 3 | 50 | 12 | sus | 0.04600 | . | 32.860 |
| 104 | 4 | 100 | 12 | sur | 0.01000 | . | 3.568 |
| 105 | 4 | 100 | 12 | sus | 0.11500 | . | 89.004 |
| 106 | 5 | 100 | 12 | sur | 0.01100 | . | 4.382 |
| 107 | 5 | 100 | 12 | sus | 0.11300 | . | 87.376 |
| 108 | 6 | 100 | 12 | sur | 0.00900 | . | 2.754 |
| 109 | 6 | 100 | 12 | sus | 0.10900 | . | 84.122 |
| 110 | 7 | 50 | 12 | sur | 0.02800 | . | 18.214 |
| 111 | 7 | 50 | 12 | sus | 0.02500 | . | 15.773 |
| 112 | 8 | 0 | 12 | sur | 0.00400 | . | -1.314 |
| 113 | 8 | 0 | 12 | sus | 0.00400 | . | -1.314 |
| 114 | 9 | 0 | 12 | sur | 0.00400 | . | -1.314 |
| 115 | 9 | 0 | 12 | sus | 0.00400 | . | -1.314 |

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AEH-12-PSEUDO-04

**Mean treatment concentration by treatment tank and sampling location (surface/suspended) for all sampling times**

The MEANS Procedure

tank=' ' loc=' '

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| . | . | . | . |

tank=1 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6159 | 1.3615 | -4.3064 | -0.9253 |

tank=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.9413 | 0.9965 | -4.1787 | -1.7040 |

tank=2 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 2.9171 | 4.3590 | -2.4954 | 8.3296 |

tank=2 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 42.1361 | 8.0878 | 32.0938 | 52.1784 |

tank=3 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 0.9643 | 4.7235 | -4.9007 | 6.8293 |

tank=3 loc=sus

| Analysis Variable : predicted_ppm Predicted |
|---|

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| Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 39.4781 | 7.0037 | 30.7819 | 48.1743 |

tank=4 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.1749 | 3.0769 | -3.9954 | 3.6457 |

tank=4 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 97.8455 | 9.0943 | 86.5534 | 109.1 |

tank=5 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.0121 | 3.8424 | -4.7831 | 4.7588 |

tank=5 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 94.0483 | 6.7196 | 85.7049 | 102.4 |

tank=6 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.3376 | 2.1833 | -3.0485 | 2.3733 |

tank=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 86.8880 | 4.0927 | 81.8063 | 91.9698 |

tank=7 loc=sur

| Analysis Variable : predicted_ppm Predicted |
|---|

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| Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 5.0326 | 8.9208 | -6.0439 | 16.1092 |

tank=7 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 34.7045 | 12.3807 | 19.3319 | 50.0772 |

tank=8 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.7786 | 1.2069 | -4.2771 | -1.2801 |

tank=8 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6159 | 1.4781 | -4.4512 | -0.7806 |

tank=9 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6159 | 1.2340 | -4.1481 | -1.0837 |

tank=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6159 | 0.9277 | -3.7678 | -1.4639 |

Performed by J. Luoma SAS version 9.3 08:07 30JAN14

Mean treatment concentration by treatment group and sampling location for all sampling times

The MEANS Procedure

thero=. loc=' '

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| . | . | . | . |

thero=0 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6701 | 1.1778 | -3.3224 | -2.0179 |

thero=0 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.7244 | 1.0859 | -3.3257 | -2.1230 |

thero=50 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 2.9713 | 6.1236 | -0.4198 | 6.3625 |

thero=50 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 38.7729 | 9.3074 | 33.6187 | 43.9272 |

thero=100 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -0.1749 | 2.88[illegible]7 | -1.7707 | 1.4210 |

thero=100 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

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| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 92.9273 | 7.9643 | 88.5168 | 97.3378 |

Performed by J. Luoma SAS version 9.3 08:07 30JAN14

Mean treatment concentration for each treatment group for each sampling time

The MEANS Procedure

thero=. time=. loc=' '

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| . | . | . | . |

thero=0 time=1 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6701 | 0.4698 | -3.8371 | -1.5031 |

thero=0 time=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -3.2126 | 0.4698 | -4.3795 | -2.0456 |

thero=0 time=3 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.9413 | 0 | . | . |

thero=0 time=3 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.3989 | 0.4698 | -3.5659 | -1.2319 |

thero=0 time=6 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -4.5687 | 0 | . | . |

thero=0 time=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -4.2975 | 0.4698 | -5.4644 | -3.1305 |

thero=0 time=9 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.8564 | 0.4698 | -3.0234 | -0.6895 |

thero=0 time=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.1277 | 0.8137 | -4.1489 | -0.1064 |

thero=0 time=12 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.3140 | 0 | . | . |

thero=0 time=12 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.5852 | 0.4698 | -2.7522 | -0.4182 |

thero=50 time=1 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.6701 | 1.2429 | -5.7577 | 0.4174 |

thero=50 time=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 52.3883 | 3.5467 | 43.5778 | 61.1989 |

thero=50 time=3 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

AEH-12-PSEUDO-04

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| -1.8564 | 0.9395 | -4.1904 | 0.4775 |

thero=50 time=3 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 39.8216 | 0.4143 | 38.7925 | 40.8508 |

thero=50 time=6 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 1.3982 | 1.2429 | -1.6893 | 4.4858 |

thero=50 time=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 35.1204 | 1.5892 | 31.1726 | 39.0683 |

thero=50 time=9 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 6.5515 | 3.2884 | -1.6174 | 14.7204 |

thero=50 time=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 37.4710 | 2.6156 | 30.9735 | 43.9685 |

thero=50 time=12 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 11.4335 | 5.8862 | -3.1887 | 26.0558 |

thero=50 time=12 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

AEH-12-PSEUDO-04

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 29.0631 | 11.8566 | -0.3902 | 58.5164 |

thero=100 time=1 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -1.5852 | 0.4698 | -2.7522 | -0.4182 |

thero=100 time=1 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 102.8 | 10.1953 | 77.5095 | 128.2 |

thero=100 time=3 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -3.2126 | 1.2429 | -6.3001 | -0.1250 |

thero=100 time=3 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 94.1568 | 5.2312 | 81.1619 | 107.2 |

thero=100 time=6 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| -2.1277 | 0 | . | . |

thero=100 time=6 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 87.6475 | 5.4177 | 74.1892 | 101.1 |

thero=100 time=9 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc |
|---|

/.EH-12-PSEUDO-04

| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|
| 2.4831 | 1.2429 | -0.6044 | 5.5707 |

thero=100 time=9 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 93.1623 | 5.4807 | 79.5476 | 106.8 |

thero=100 time=12 loc=sur

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 3.5680 | 0.8137 | 1.5468 | 5.5893 |

thero=100 time=12 loc=sus

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 86.8338 | 2.4858 | 80.6587 | 93.0089 |

Performed by J. Luoma SAS version 9.3 08:07 30JAN14

Mean concentration for standard checks for all sampling times

The MEANS Procedure

thero=25

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 25.8084 | 0.4698 | 24.6414 | 26.9754 |

thero=50

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 48.8624 | 0.4698 | 47.6955 | 50.0294 |

thero=100

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 95.5130 | 5.6373 | 81.5092 | 109.5 |

thero=200

| Analysis Variable : predicted_ppm Predicted Value of conc | | | |
|---|---|---|---|
| Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
| 194.2 | 1.8791 | 189.6 | 198.9 |

Performed by J. Luoma SAS version 9.3 08:07 30JAN14 JL

File Folder: 14c Item Number: 4

# Appendix 8. Survival Assessment Summary

| Item Number | Item Description | Number of Pages | Report Page Number |
|---|---|---|---|
| 1 | Zebra Mussel Survival on Artificial Substrate – Lake Carlos – Whole Tank – Data Summary | 2 | 437 |
| 2 | Zebra Mussel Survival on Artificial Substrate – Lake Shawano – Whole Tank – Data Summary | 2 | 439 |
| 3 | Zebra Mussel Survival on Artificial Substrate – Lake Carlos – Bottom Injection –Data Summary | 2 | 441 |
| 4 | Zebra Mussel Survival on Artificial Substrate – Lake Shawano – Bottom Injection – Data Summary | 2 | 443 |
| 5 | Zebra Mussel Survival on Artificial Substrate – All Exposures – Data Summary | 6 | 445 |

| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (pages 29)<br>Data Source: File Folder: 9d<br>Forms: "Zebra Mussel Survival"<br><br>File Name: See filenames as stated below | Action | Date | Initials |
|---|---|---|---|
| | Created...... | 28-Oct-13 | KLW TJS |
| | Revised...... | 4-Mar-14 | TJS TJS |
| | Reviewed... | 4MAR14 | TJS |
| | Certified... | 3/4/14 | JA |

## Zebra Mussel Survival on Artificial Substrate

Test Article: MBI 401 SDP (*Pseudomonas fluorescens Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Whole Tank

Data Explanation:
Each tank replicate contained 9 perforated aluminum trays (~15.2 cm x 15.2 cm with 2.5 cm sides) with attached zebra mussels enclosed in a mesh containment bag (~20.3 x 25.4 x 5.1 cm; 0.31 x 0.31 cm openings). Each substrate contained a minimum of 50 adhering zebra mussels prior to treatment. Aluminum trays were coded with tags according to treatment type (W = whole water body, B = bottom injection; tank number 1-9, row letter A, B, C; position number 1-3). Example code of W 1A3 = whole water body (tank) treatment, Tank 1, Row A, position 3. The tanks are numbered 1-9; the rows are in order from the front of the tank (isle) to the rear of the tank (wall) (A = isle; B = middle; C = wall); the position is from left to right in order (1 = left; 2 = center; 3 = right). Three trays were removed after 6, 9 and 12 h of exposure. The substrates were consolidated into wire mesh holding cages with 1 sampling time period per cage and the treatment levels indiscriminately distributed. The wire mesh holding cages were held in the lake until survival assessments were completed at 28 days post-dosing termination.

Survival Data Filename:
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Survival (Whole Tank).xlsx]Survival Data

Data anomalies and deviations:
NONE

**File Folder:** 9d

Item Number 1
Page 1 of 2

## Zebra Mussel Survival on Artificial Substrate

| Treatment Level (mg/L) | 6 hour Sample Time | | | | 9 hour Sample Time | | | | 12 hour Sample Time | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample ID | Total Number of Animals | Number Alive | Number Dead | Sample ID | Total Number of Animals | Number Alive | Number Dead | Sample ID | Total Number of Animals | Number Alive | Number Dead |
| 0 | W2A1 | 339 | 337 | 2 | W2A3 | 326 | 320 | 6 | W2A2 | 390 | 374 | 16 |
| | W2B2 | 237 | 227 | 10 | W2B3 | 236 | 230 | 6 | W2B1 | 185 | 181 | 4 |
| | W2C1 | 168 | 165 | 3 | W2C3 | 226 | 220 | 6 | W2C2 | 198 | 190 | 8 |
| | W3A1 | 328 | 305 | 23 | W3A3 | 178 | 171 | 7 | W3A2 | 185 | 180 | 5 |
| | W3B1 | 255 | 252 | 3 | W3B3 | 213 | 211 | 2 | W3B2 | 312 | 304 | 8 |
| | W3C3 | 310 | 304 | 6 | W3C2 | 214 | 203 | 11 | W3C1 | 259 | 254 | 5 |
| | W5A1 | 269 | 264 | 5 | W5A3 | 177 | 175 | 2 | W5A2 | 240 | 238 | 2 |
| | W5B2 | 173 | 171 | 2 | W5B3 | 192 | 185 | 7 | W5B1 | 196 | 190 | 6 |
| | W5C1 | 175 | 175 | 0 | W5C3 | 173 | 162 | 11 | W5C2 | 212 | 206 | 6 |
| 50 | W1A2 | 280 | 6 | 274 | W1A1 | 164 | 3 | 161 | W1C2 | 175 | 0 | 175 |
| | W1A3 | 149 | 0 | 149 | W1B1 | 128 | 5 | 123 | W1B3 | 160 | 1 | 159 |
| | W1B2 | 232 | 4 | 228 | W1C1 | 267 | 0 | 267 | W1C3 | 266 | 0 | 266 |
| | W4B2 | 214 | 4 | 210 | W4A1 | 221 | 0 | 221 | W4A2 | 158 | 3 | 155 |
| | W4C1 | 191 | 0 | 191 | W4A3 | 375 | 0 | 375 | W4B1 | 280 | 3 | 277 |
| | W4C2 | 175 | 4 | 171 | W4B3 | 265 | 4 | 261 | W4C3 | 455 | 2 | 453 |
| | W8B1 | 208 | 5 | 203 | W8A3 | 131 | 4 | 127 | W8A1 | 151 | 1 | 150 |
| | W8B2 | 178 | 1 | 177 | W8C1 | 169 | 0 | 169 | W8A2 | 119 | 0 | 119 |
| | W8B3 | 248 | 1 | 247 | W8C3 | 180 | 0 | 180 | W8C2 | 290 | 3 | 287 |
| 100 | W6A2 | 257 | 7 | 250 | W6B2 | 188 | 1 | 187 | W6A1 | 239 | 3 | 236 |
| | W6A3 | 235 | 8 | 227 | W6B3 | 183 | 2 | 181 | W6B1 | 249 | 1 | 248 |
| | W6C3 | 267 | 3 | 264 | W6C1 | 176 | 0 | 176 | W6C2 | 155 | 2 | 153 |
| | W7A1 | 212 | 2 | 210 | W7A3 | 258 | 3 | 255 | W7A2 | 237 | 1 | 236 |
| | W7B2 | 266 | 10 | 256 | W7B3 | 193 | 0 | 193 | W7B1 | 286 | 0 | 286 |
| | W7C1 | 269 | 2 | 267 | W7C3 | 158 | 3 | 155 | W7C2 | 203 | 0 | 203 |
| | W9A1 | 283 | 8 | 275 | W9A3 | 393 | 1 | 392 | W9A2 | 316 | 0 | 316 |
| | W9B2 | 190 | 2 | 188 | W9B3 | 128 | 0 | 128 | W9B1 | 273 | 5 | 268 |
| | W9C1 | 324 | 7 | 317 | W9C3 | 260 | 0 | 260 | W9C2 | 156 | 1 | 155 |

File Folder: 9d

Item Number 1
Page 2 of 2

| Study Number: AEH-12-PSEUDO-04<br>Electronic Lab Notebook (pages 29)<br>Data Source: File Folder: 11d<br>Forms: "Zebra Mussel Survival"<br><br>File Name: See filenames as stated below | Action | Date | Initials |
|---|---|---|---|
| | Created...... | 28-Oct-13 | KLW TJS |
| | Revised...... | 4-Mar-14 | TJS TJS |
| | Reviewed... | 4MAR14 | TJS |
| | Certified... | 3/4/14 | JA |

## Zebra Mussel Survival on Artificial Substrate

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

Data Explanation:
Each tank replicate contained 9 perforated aluminum trays (~15.2 cm x 15.2 cm with 2.5 cm sides) with attached zebra mussels enclosed in a mesh containment bag (~20.3 x 25.4 x 5.1 cm; 0.31 x 0.31 cm openings). Each substrate contained a minimum of 50 adhering zebra mussels prior to treatment. Aluminum trays were coded with tags according to treatment type (W = whole water body, B = bottom injection; tank number 1-9, row letter A, B, C; position number 1-3). Example code of W 1A3 = whole water body (tank) treatment, Tank 1, Row A, position 3. The tanks are numbered 1-9; the rows are in order from the front of the tank (isle) to the rear of the tank (wall) (A = isle; B = middle; C = wall); the position is from left to right in order (1 = left; 2 = center; 3 = right). Three trays were removed after 6, 9 and 12 h of expsoure. The substrates were consolidated into wire mesh holding cages with 1 sampling time period per cage and the treatment levels indiscriminately distributed. The wire mesh holding cages were held in the lake until survival assessments were completed at 34 days post-dosing termination.

Survival Data Filename:
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Survival (Whole Tank).xlsx]Survival Data

Data anomalies and deviations:
NONE

File Folder: 11d

Item Number 1
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 29)
Data Source: File Folder: 11d
Forms: "Zebra Mussel Survival"

Test Article: MBI 401 SDP (Pf-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 6, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Whole Tank

## Zebra Mussel Survival on Artificial Substrate

| Treatment Level (mg/L) | 6 hour Sample Time | | | | 9 hour Sample Time | | | | 12 hour Sample Time | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample ID | Total Number of Animals | Number Alive | Number Dead | Sample ID | Total Number of Animals | Number Alive | Number Dead | Sample ID | Total Number of Animals | Number Alive | Number Dead |
| | W1A1 | 109 | 105 | 4 | W1A3 | 159 | 150 | 9 | W1A2 | 101 | 96 | 5 |
| | W1B1 | 118 | 111 | 7 | W1B3 | 84 | 82 | 2 | W1C2 | 108 | 102 | 6 |
| | W1B2 | 87 | 85 | 2 | W1C1 | 144 | 138 | 6 | W1C3 | 111 | 106 | 5 |
| | W4A1 | 64 | 61 | 3 | W4A3 | 110 | 103 | 7 | W4B3 | 79 | 76 | 3 |
| 0 | W4A2 | 74 | 67 | 7 | W4B2 | 75 | 72 | 3 | W4C2 | 81 | 77 | 4 |
| | W4B1 | 78 | 74 | 4 | W4C1 | 71 | 69 | 2 | W4C3 | 141 | 137 | 4 |
| | W7A1 | 124 | 122 | 2 | W7A3 | 54 | 52 | 2 | W7A2 | 92 | 90 | 2 |
| | W7B1 | 80 | 78 | 2 | W7B2 | 108 | 102 | 6 | W7B3 | 104 | 102 | 2 |
| | W7C1 | 108 | 103 | 5 | W7C3 | 111 | 105 | 6 | W7C2 | 90 | 87 | 3 |
| | W2A1 | 108 | 17 | 91 | W2A3 | 114 | 26 | 88 | W2A2 | 98 | 1 | 97 |
| | W2B1 | 116 | 10 | 106 | W2B2 | 103 | 9 | 94 | W2B3 | 85 | 5 | 80 |
| | W2C1 | 61 | 4 | 57 | W2C3 | 117 | 5 | 112 | W2C2 | 86 | 0 | 86 |
| | W5A1 | 95 | 8 | 87 | W5A3 | 61 | 2 | 59 | W5A2 | 60 | 0 | 60 |
| 50 | W5B1 | 67 | 8 | 59 | W5B2 | 105 | 22 | 83 | W5B3 | 63 | 5 | 58 |
| | W5C1 | 85 | 14 | 71 | W5C3 | 80 | 19 | 61 | W5C2 | 114 | 2 | 112 |
| | W8A3 | 96 | 5 | 91 | W8A1 | 148 | 2 | 146 | W8A2 | 76 | 3 | 73 |
| | W8B3 | 149 | 57 | 92 | W8B1 | 85 | 5 | 80 | W8B2 | 88 | 3 | 85 |
| | W8C1 | 136 | 3 | 133 | W8C2 | 61 | 1 | 60 | W8C3 | 129 | 1 | 128 |
| | W3A1 | 138 | 27 | 111 | W3B1 | 51 | 2 | 49 | W3A2 | 95 | 1 | 94 |
| | W3B2 | 122 | 8 | 114 | W3C2 | 73 | 4 | 69 | W3A3 | 131 | 6 | 125 |
| | W3B3 | 104 | 9 | 95 | W3C3 | 88 | 0 | 88 | W3C1 | 104 | 1 | 103 |
| | W6A1 | 69 | 0 | 69 | W6B1 | 59 | 0 | 59 | W6B3 | 77 | 0 | 77 |
| 100 | W6A2 | 124 | 3 | 121 | W6B2 | 94 | 13 | 81 | W6C1 | 62 | 3 | 59 |
| | W6A3 | 57 | 3 | 54 | W6C2 | 94 | 0 | 94 | W6C3 | 102 | 1 | 101 |
| | W9A1 | 77 | 2 | 75 | W9A3 | 107 | 14 | 93 | W9A2 | 104 | 0 | 104 |
| | W9B1 | 119 | 4 | 115 | W9B2 | 69 | 0 | 69 | W9B3 | 102 | 2 | 100 |
| | W9C1 | 118 | 19 | 99 | W9C3 | 123 | 7 | 116 | W9C2 | 60 | 2 | 58 |

File Folder: 11d

Item Number 1
Page 2 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 29)
Data Source: File Folder: 12d
Forms: "Zebra Mussel Survival"

File Name: See filenames as stated below

| Action | Date | Initials |
|---|---|---|
| Created...... | 28-Oct-13 | KLW TJS |
| Revised...... | 4-Mar-14 | TJS TJS |
| Reviewed... | 4MAR14 | TJS |
| Certified... | 3/4/14 | JK |

## Zebra Mussel Survival on Artificial Substrate

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

Data Explanation:

Each tank replicate contained 3 perforated aluminum trays (~15.2 cm x 15.2 cm with 2.5 cm sides) with attached zebra mussels enclosed in a mesh containment bag (~20.3 x 25.4 x 5.1 cm; 0.31 x 0.31 cm openings). Each substrate contained a minimum of 50 adhering zebra mussels prior to treatment. Aluminum trays were coded with tags according to treatment type (W = whole water body, B = bottom injection; tank number 1-9, row letter A, B, C; position number 1-3). Example code of W 1A3 = whole water body (tank) treatment, Tank 1, Row A, position 3. The tanks are numbered 1-9; the rows are in order from the front of the tank (isle) to the rear of the tank (wall) (A = isle; B = middle; C = wall); the position is from left to right in order (1 = left; 2 = center; 3 = right). All substrates were removed after 12 h of exposure. The substrates were consolidated into a wire mesh holding cage with the treatment levels indiscriminately distributed. The wire mesh holding cages were held in the lake until survival assessments were completed at 27 days post-dosing termination.

Survival Data Filename:
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Carlos Survival (Bottom Injection).xlsx]Survival Data

Data anomalies and deviations:
NONE

File Folder: 12d

Item Number 1
Page 1 of 2

Study Number: AEH-12-PSEUDO-04
Electronic Lab Notebook (pages 29)
Data Source: File Folder: 12d
Forms "Zebra Mussel Survival"

Test Article: MBI 401 SDP (Pf-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 17, 2012
Test Location: Lake Carlos, Alexandria, MN
Treatment Type: Bottom Injection

## Zebra Mussel Survival on Artificial Substrate

| Treatment Level (mg/L) | Sample ID | Total Number of Animals | Number Alive | Number Dead |
|---|---|---|---|---|
| 0 | B3A3 | 196 | 192 | 4 |
| | B3B1 | 242 | 237 | 5 |
| | B3C3 | 253 | 246 | 7 |
| | B6A2 | 186 | 182 | 4 |
| | B6B1 | 217 | 206 | 11 |
| | B6C1 | 179 | 175 | 4 |
| | B7A1 | 225 | 221 | 4 |
| | B7B3 | 176 | 167 | 9 |
| | B7C1 | 276 | 273 | 3 |
| 50 | B1A1 | 210 | 52 | 158 |
| | B1B2 | 510 | 86 | 424 |
| | B1B3 | 279 | 62 | 217 |
| | B4B1 | 188 | 10 | 178 |
| | B4B2 | 256 | 65 | 191 |
| | B4B3 | 188 | 14 | 174 |
| | B8A3 | 282 | 50 | 232 |
| | B8B3 | 291 | 69 | 222 |
| | B8C2 | 200 | 39 | 161 |
| 100 | B2A1 | 279 | 18 | 261 |
| | B2B3 | 153 | 21 | 132 |
| | B2C1 | 193 | 39 | 154 |
| | B5A1 | 329 | 112 | 217 |
| | B5B3 | 393 | 121 | 272 |
| | B5C1 | 264 | 55 | 209 |
| | B9A1 | 269 | 13 | 256 |
| | B9B3 | 179 | 40 | 139 |
| | B9C1 | 288 | 25 | 263 |

File Folder: 12d

Item Number 1
Page 2 of 2

| Study Number: AEH-12-PSEUDO-04 | Action | Date | Initials |
|---|---|---|---|
| Electronic Lab Notebook (pages 29 - 30) | Created...... | 28-Oct-13 | KLW TJS |
| Data Source: File Folder: 14d | Revised...... | 4-Mar-14 | TJS TJS |
| Forms: "Zebra Mussel Survival" | Reviewed... | 4MAR14 | TJS |
| | Certified... | 3/4/14 | JA |
| File Name: See filenames as stated below | | | |

**Zebra Mussel Survival on Artificial Substrate**

Test Article: MBI 401 SDP [*Pseudomonas fluorescens Pf*-CL 145A (SDP)]
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: September 8, 2012
Test Location: Lake Shawano, Shawano, WI
Treatment Type: Bottom Injection

Data Explanation:

Each tank replicate contained 4 perforated aluminum trays (~15.2 cm x 15.2 cm with 2.5 cm sides) with attached zebra mussels enclosed in a mesh containment bag (~20.3 x 25.4 x 5.1 cm; 0.31 x 0.31 cm openings). Each substrate contained a minimum of 50 adhering zebra mussels prior to treatment. Aluminum trays were coded with tags according to treatment type (W = whole water body, B = bottom injection; tank number 1-9, row letter A, B, C; position number 1-3). Example code of W 1A3 = whole water body (tank) treatment, Tank 1, Row A, position 3. The tanks are numbered 1-9; the rows are in order from the front of the tank (isle) to the rear of the tank (wall) (A = isle; B = middle; C = wall); the position is from left to right in order (1 = left; 2 = center; 3 = right). All substrates were removed after 12 h of exposure. The substrates were consolidated into a wire mesh holding cage with the treatment levels indiscriminately distributed. The wire mesh holding cages were held in the lake until survival assessments were completed at 32 days post-exposure termination.

Survival Data Filename:
I:\AEH-12-PSEUDO-04\Data Summaries\[Lake Shawano Survival (Bottom Injection).xlsx]Survival Data

Data anomalies and deviations:
NONE

**File Folder:** 14d

Item Number 1

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## Zebra Mussel Survival on Artificial Substrate

| Treatment Level (mg/L) | Sample ID | Total Number of Animals | Number Alive | Number Dead |
|---|---|---|---|---|
| 0 | B1A3 | 107 | 100 | 7 |
| | B1C1 | 137 | 127 | 10 |
| | B1C2 | 111 | 108 | 3 |
| | B1C3 | 147 | 139 | 8 |
| | B8A3 | 140 | 133 | 7 |
| | B8B1 | 81 | 76 | 5 |
| | B8B2 | 134 | 128 | 6 |
| | B8B3 | 163 | 159 | 4 |
| | B9A2 | 115 | 115 | 0 |
| | B9B1 | 84 | 80 | 4 |
| | B9B2 | 87 | 85 | 2 |
| | B9B3 | 137 | 127 | 10 |
| 50 | B2A2 | 119 | 4 | 115 |
| | B2B1 | 105 | 3 | 102 |
| | B2B2 | 116 | 2 | 114 |
| | B2B3 | 145 | 5 | 140 |
| | B3A2 | 117 | 4 | 113 |
| | B3B1 | 144 | 8 | 136 |
| | B3B2 | 103 | 0 | 103 |
| | B3C2 | 109 | 2 | 107 |
| | B7A2 | 115 | 2 | 113 |
| | B7B1 | 128 | 4 | 124 |
| | B7B2 | 116 | 3 | 113 |
| | B7B3 | 109 | 6 | 103 |
| 100 | B4A2 | 117 | 1 | 116 |
| | B4B2 | 123 | 1 | 122 |
| | B4B3 | 126 | 2 | 124 |
| | B4C3 | 104 | 0 | 104 |
| | B5A2 | 106 | 2 | 104 |
| | B5B1 | 105 | 0 | 105 |
| | B5B2 | 97 | 0 | 97 |
| | B5B3 | 108 | 0 | 108 |
| | B6B2 | 121 | 2 | 119 |
| | B6B3 | 113 | 0 | 113 |
| | B6C2 | 127 | 1 | 126 |
| | B6C3 | 98 | 3 | 95 |

File Folder: 14d

Item Number 1
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| Study Number: AEH-12-PSEUDO-04 | | Action | Date | Initials |
|---|---|---|---|---|
| Electronic Lab Notebook (pages 29-30) | | Created...... | 14-Feb-13 | TJS TJS |
| Data Source: File Folder: 18 | | Revised...... | 4-Mar-14 | TJS TJS |
| Forms: "Zebra Mussel Survival" | | Reviewed... | 4MAR14 | TJS |
| | | Certified... | 3/4/14 | JK |
| File Name: See filenames as stated below | | | | |

**Zebra Mussel Survival on Artificial Substrate**

Test Article: MBI 401 SDP (*Pseudomonas fluorescens Pf*-CL 145A (SDP))
Article Lot #: 401P12163C and 401P12164C Mix
Exposure Date: August 15 and 17, and September 6 and 8, 2012
Test Locations: Lake Carlos, Alexandria, MN and Lake Shawano, Shawano, WI
Treatment Type: Whole Tank and Bottom Injection

Data Explanation:
Each tank replicate contained 3, 4, or 9 perforated aluminum trays (~15.2 cm x 15.2 cm with 2.5 cm sides), depending on lake and treatment type, with attached zebra mussels enclosed in a mesh containment bag (~20.3 x 25.4 x 5.1 cm; 0.31 x 0.31 cm openings). Each substrate contained a minimum of 50 adhering zebra mussels prior to treatment. Aluminum trays were coded with tags according to treatment type (W = whole water body, B = bottom injection; tank number 1-9, row letter A, B, C; position number 1-3). Example code of W 1A3 = whole water body (tank) treatment, Tank 1, Row A, position 3. The tanks are numbered 1-9; the rows are in order from the front of the tank (isle) to the rear of the tank (wall) (A = isle; B = middle; C = wall); the position is from left to right in order (1 = left; 2 = center; 3 = right). For whole water body treatments, three trays were removed after 6, 9 and 12 h of exposure. The substrates were consolidated into wire mesh holding cages with 1 sampling time period per cage and the treatment levels indiscriminately distributed. The wire mesh holding cages were held in the lake until survival assessments were completed at 28 days post-dosing termination.

Survival Data for SAS Filename:
I:\AEH-12-PSEUDO-04\Data Summaries\[All Exposures Survival Assessment.xlsx]Survival Data for SAS
- loc = Location
  - LC = Lake Carlos
  - SL = Shawano Lake
- trt_typ = Treatment Group
  - WT = Whole Tank
  - BI = Bottom Injection
- conc = Concentration
  - 0 = control
  - 50 = 50 mg/L (A.I.) treatment
  - 100 = 100 mg/L (A.I.) treatment
- time = Exposure Duration
- tnk = Exposure Tank ID
- tray = Substrate ID
- tot = Total Number of Zebra Mussels
- dead = Number of Zebra Mussel Mortalities

Data anomalies and deviations:
NONE

**File Folder:** 18

Item Number 1
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| loc | trt_typ | conc | time | tnk | tray | tot | dead |
|---|---|---|---|---|---|---|---|
| LC | WT | 100 | 6 | 7 | B2 | 266 | 256 |
| LC | WT | 100 | 6 | 9 | B2 | 190 | 188 |
| LC | WT | 50 | 6 | 1 | A3 | 149 | 149 |
| LC | WT | 0 | 6 | 5 | C1 | 175 | 0 |
| LC | WT | 0 | 6 | 2 | C1 | 168 | 3 |
| LC | WT | 100 | 6 | 6 | C3 | 267 | 264 |
| LC | WT | 0 | 6 | 2 | B2 | 237 | 10 |
| LC | WT | 100 | 6 | 7 | A1 | 212 | 210 |
| LC | WT | 0 | 6 | 3 | A1 | 328 | 23 |
| LC | WT | 100 | 6 | 6 | A3 | 235 | 227 |
| LC | WT | 50 | 6 | 4 | C2 | 175 | 171 |
| LC | WT | 0 | 6 | 5 | B2 | 173 | 2 |
| LC | WT | 0 | 6 | 3 | B1 | 255 | 3 |
| LC | WT | 50 | 6 | 8 | B1 | 208 | 203 |
| LC | WT | 50 | 6 | 1 | A2 | 280 | 274 |
| LC | WT | 100 | 6 | 6 | A2 | 257 | 250 |
| LC | WT | 0 | 6 | 2 | A1 | 339 | 2 |
| LC | WT | 50 | 6 | 8 | B2 | 178 | 177 |
| LC | WT | 100 | 6 | 9 | C1 | 324 | 317 |
| LC | WT | 100 | 6 | 7 | C1 | 269 | 267 |
| LC | WT | 50 | 6 | 4 | B2 | 214 | 210 |
| LC | WT | 50 | 6 | 1 | B2 | 232 | 228 |
| LC | WT | 0 | 6 | 5 | A1 | 269 | 5 |
| LC | WT | 0 | 6 | 3 | C3 | 310 | 6 |
| LC | WT | 50 | 6 | 4 | C1 | 191 | 191 |
| LC | WT | 100 | 6 | 9 | A1 | 283 | 275 |
| LC | WT | 50 | 6 | 8 | B3 | 248 | 247 |
| LC | WT | 0 | 9 | 5 | B3 | 192 | 7 |
| LC | WT | 0 | 9 | 3 | A3 | 178 | 7 |
| LC | WT | 0 | 9 | 5 | C3 | 173 | 11 |
| LC | WT | 50 | 9 | 4 | B3 | 265 | 261 |
| LC | WT | 50 | 9 | 4 | A3 | 375 | 375 |
| LC | WT | 100 | 9 | 6 | B2 | 188 | 187 |
| LC | WT | 0 | 9 | 2 | C3 | 226 | 6 |
| LC | WT | 100 | 9 | 7 | B3 | 193 | 193 |
| LC | WT | 0 | 9 | 3 | B3 | 213 | 2 |
| LC | WT | 50 | 9 | 8 | C1 | 169 | 169 |
| LC | WT | 50 | 9 | 1 | A1 | 164 | 161 |
| LC | WT | 100 | 9 | 7 | C3 | 158 | 155 |
| LC | WT | 50 | 9 | 4 | A1 | 221 | 221 |
| LC | WT | 0 | 9 | 3 | C2 | 214 | 11 |
| LC | WT | 50 | 9 | 8 | C3 | 180 | 180 |
| LC | WT | 100 | 9 | 6 | C1 | 176 | 176 |
| LC | WT | 50 | 9 | 8 | A3 | 131 | 127 |
| LC | WT | 100 | 9 | 9 | A3 | 393 | 392 |
| LC | WT | 100 | 9 | 9 | B3 | 128 | 128 |
| LC | WT | 100 | 9 | 6 | B3 | 183 | 181 |
| LC | WT | 50 | 9 | 1 | B1 | 128 | 123 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| LC | WT | 100 | 9 | 9 | C3 | 260 | 260 |
| LC | WT | 0 | 9 | 2 | B3 | 236 | 6 |
| LC | WT | 0 | 9 | 5 | A3 | 177 | 2 |
| LC | WT | 100 | 9 | 7 | A3 | 258 | 255 |
| LC | WT | 0 | 9 | 2 | A3 | 326 | 6 |
| LC | WT | 50 | 9 | 1 | C1 | 267 | 267 |
| LC | WT | 0 | 12 | 3 | B2 | 312 | 8 |
| LC | WT | 0 | 12 | 3 | A2 | 185 | 5 |
| LC | WT | 100 | 12 | 6 | B1 | 249 | 248 |
| LC | WT | 50 | 12 | 1 | C3 | 266 | 266 |
| LC | WT | 100 | 12 | 6 | A1 | 239 | 236 |
| LC | WT | 0 | 12 | 2 | A2 | 390 | 16 |
| LC | WT | 50 | 12 | 1 | C2 | 175 | 175 |
| LC | WT | 50 | 12 | 1 | B3 | 160 | 159 |
| LC | WT | 0 | 12 | 3 | C1 | 259 | 5 |
| LC | WT | 0 | 12 | 2 | B1 | 185 | 4 |
| LC | WT | 50 | 12 | 8 | C2 | 290 | 287 |
| LC | WT | 100 | 12 | 9 | A2 | 316 | 316 |
| LC | WT | 0 | 12 | 2 | C2 | 198 | 8 |
| LC | WT | 100 | 12 | 7 | A2 | 237 | 236 |
| LC | WT | 50 | 12 | 8 | A2 | 119 | 119 |
| LC | WT | 100 | 12 | 9 | B1 | 273 | 268 |
| LC | WT | 50 | 12 | 8 | A1 | 151 | 150 |
| LC | WT | 0 | 12 | 5 | A2 | 240 | 2 |
| LC | WT | 0 | 12 | 5 | C2 | 212 | 6 |
| LC | WT | 100 | 12 | 7 | B1 | 286 | 286 |
| LC | WT | 0 | 12 | 5 | B1 | 196 | 6 |
| LC | WT | 50 | 12 | 4 | A2 | 158 | 155 |
| LC | WT | 100 | 12 | 7 | C2 | 203 | 203 |
| LC | WT | 100 | 12 | 9 | C2 | 156 | 155 |
| LC | WT | 50 | 12 | 4 | C3 | 455 | 453 |
| LC | WT | 50 | 12 | 4 | B1 | 280 | 277 |
| LC | WT | 100 | 12 | 6 | C2 | 155 | 153 |
| LC | BI | 100 | 12 | 9 | A1 | 269 | 256 |
| LC | BI | 50 | 12 | 4 | B2 | 256 | 191 |
| LC | BI | 100 | 12 | 9 | B3 | 179 | 139 |
| LC | BI | 100 | 12 | 2 | B3 | 153 | 132 |
| LC | BI | 50 | 12 | 1 | B3 | 279 | 217 |
| LC | BI | 0 | 12 | 6 | C1 | 179 | 4 |
| LC | BI | 50 | 12 | 8 | C2 | 200 | 161 |
| LC | BI | 0 | 12 | 7 | A1 | 225 | 4 |
| LC | BI | 50 | 12 | 1 | B2 | 510 | 424 |
| LC | BI | 50 | 12 | 4 | B1 | 188 | 178 |
| LC | BI | 100 | 12 | 2 | C1 | 193 | 154 |
| LC | BI | 100 | 12 | 9 | C1 | 288 | 263 |
| LC | BI | 0 | 12 | 3 | C3 | 253 | 7 |
| LC | BI | 50 | 12 | 4 | B3 | 188 | 174 |
| LC | BI | 50 | 12 | 8 | B3 | 291 | 222 |
| LC | BI | 100 | 12 | 5 | A1 | 329 | 217 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| LC | BI | 0 | 12 | 7 | B3 | 176 | 9 |
| LC | BI | 0 | 12 | 3 | B1 | 242 | 5 |
| LC | BI | 0 | 12 | 6 | B1 | 217 | 11 |
| LC | BI | 100 | 12 | 5 | B3 | 393 | 272 |
| LC | BI | 0 | 12 | 6 | A2 | 186 | 4 |
| LC | BI | 50 | 12 | 8 | A3 | 282 | 232 |
| LC | BI | 0 | 12 | 3 | A3 | 196 | 4 |
| LC | BI | 50 | 12 | 1 | A1 | 210 | 158 |
| LC | BI | 0 | 12 | 7 | C1 | 276 | 3 |
| LC | BI | 100 | 12 | 2 | A1 | 279 | 261 |
| LC | BI | 100 | 12 | 5 | C1 | 264 | 209 |
| SL | WT | 50 | 6 | 8 | C1 | 136 | 133 |
| SL | WT | 0 | 6 | 7 | B1 | 80 | 2 |
| SL | WT | 100 | 6 | 9 | A1 | 77 | 75 |
| SL | WT | 0 | 6 | 1 | A1 | 109 | 4 |
| SL | WT | 0 | 6 | 1 | B2 | 87 | 2 |
| SL | WT | 0 | 6 | 7 | C1 | 108 | 5 |
| SL | WT | 100 | 6 | 6 | A3 | 57 | 54 |
| SL | WT | 100 | 6 | 9 | C1 | 118 | 99 |
| SL | WT | 100 | 6 | 3 | B3 | 104 | 95 |
| SL | WT | 50 | 6 | 8 | A3 | 96 | 91 |
| SL | WT | 100 | 6 | 3 | B2 | 122 | 114 |
| SL | WT | 100 | 6 | 6 | A2 | 124 | 121 |
| SL | WT | 50 | 6 | 2 | A1 | 108 | 91 |
| SL | WT | 0 | 6 | 1 | B1 | 118 | 7 |
| SL | WT | 50 | 6 | 5 | B1 | 67 | 59 |
| SL | WT | 100 | 6 | 3 | A1 | 138 | 111 |
| SL | WT | 100 | 6 | 9 | B1 | 119 | 115 |
| SL | WT | 0 | 6 | 4 | A1 | 64 | 3 |
| SL | WT | 50 | 6 | 2 | B1 | 116 | 106 |
| SL | WT | 0 | 6 | 7 | A1 | 124 | 2 |
| SL | WT | 100 | 6 | 6 | A1 | 69 | 69 |
| SL | WT | 50 | 6 | 5 | C1 | 85 | 71 |
| SL | WT | 50 | 6 | 2 | C1 | 61 | 57 |
| SL | WT | 0 | 6 | 4 | B1 | 78 | 4 |
| SL | WT | 0 | 6 | 4 | A2 | 74 | 7 |
| SL | WT | 50 | 6 | 5 | A1 | 95 | 87 |
| SL | WT | 50 | 6 | 8 | B3 | 149 | 92 |
| SL | WT | 100 | 9 | 3 | B1 | 51 | 49 |
| SL | WT | 50 | 9 | 8 | C2 | 61 | 60 |
| SL | WT | 100 | 9 | 3 | C2 | 73 | 69 |
| SL | WT | 0 | 9 | 4 | C1 | 71 | 2 |
| SL | WT | 0 | 9 | 1 | B3 | 84 | 2 |
| SL | WT | 0 | 9 | 1 | A3 | 159 | 9 |
| SL | WT | 50 | 9 | 8 | A1 | 148 | 146 |
| SL | WT | 0 | 9 | 7 | B2 | 108 | 6 |
| SL | WT | 50 | 9 | 2 | C3 | 117 | 112 |
| SL | WT | 100 | 9 | 9 | C3 | 123 | 116 |
| SL | WT | 0 | 9 | 7 | A3 | 54 | 2 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SL | WT | 0 | 9 | 7 | C3 | 111 | 6 |
| SL | WT | 50 | 9 | 8 | B1 | 85 | 80 |
| SL | WT | 50 | 9 | 5 | A3 | 61 | 59 |
| SL | WT | 100 | 9 | 3 | C3 | 88 | 88 |
| SL | WT | 50 | 9 | 2 | B2 | 103 | 94 |
| SL | WT | 100 | 9 | 9 | A3 | 107 | 93 |
| SL | WT | 100 | 9 | 6 | B1 | 59 | 59 |
| SL | WT | 50 | 9 | 5 | C3 | 80 | 61 |
| SL | WT | 100 | 9 | 9 | B2 | 69 | 69 |
| SL | WT | 50 | 9 | 2 | A3 | 114 | 88 |
| SL | WT | 100 | 9 | 6 | C2 | 94 | 94 |
| SL | WT | 0 | 9 | 4 | B2 | 75 | 3 |
| SL | WT | 100 | 9 | 6 | B2 | 94 | 81 |
| SL | WT | 50 | 9 | 5 | B2 | 105 | 83 |
| SL | WT | 0 | 9 | 4 | A3 | 110 | 7 |
| SL | WT | 0 | 9 | 1 | C1 | 144 | 6 |
| SL | WT | 100 | 12 | 6 | C1 | 62 | 59 |
| SL | WT | 100 | 12 | 3 | A3 | 131 | 125 |
| SL | WT | 50 | 12 | 5 | C2 | 114 | 112 |
| SL | WT | 50 | 12 | 2 | A2 | 98 | 97 |
| SL | WT | 0 | 12 | 1 | A2 | 101 | 5 |
| SL | WT | 0 | 12 | 1 | C2 | 108 | 6 |
| SL | WT | 100 | 12 | 3 | A2 | 95 | 94 |
| SL | WT | 50 | 12 | 8 | B2 | 88 | 85 |
| SL | WT | 100 | 12 | 9 | C2 | 60 | 58 |
| SL | WT | 100 | 12 | 6 | B3 | 77 | 77 |
| SL | WT | 0 | 12 | 7 | A2 | 92 | 2 |
| SL | WT | 50 | 12 | 2 | C2 | 86 | 86 |
| SL | WT | 100 | 12 | 6 | C3 | 102 | 101 |
| SL | WT | 50 | 12 | 8 | C3 | 129 | 128 |
| SL | WT | 100 | 12 | 3 | C1 | 104 | 103 |
| SL | WT | 50 | 12 | 2 | B3 | 85 | 80 |
| SL | WT | 50 | 12 | 5 | B3 | 63 | 58 |
| SL | WT | 0 | 12 | 4 | C3 | 141 | 4 |
| SL | WT | 0 | 12 | 7 | C2 | 90 | 3 |
| SL | WT | 0 | 12 | 7 | B3 | 104 | 2 |
| SL | WT | 50 | 12 | 8 | A2 | 76 | 73 |
| SL | WT | 0 | 12 | 4 | B3 | 79 | 3 |
| SL | WT | 100 | 12 | 9 | B3 | 102 | 100 |
| SL | WT | 0 | 12 | 4 | C2 | 81 | 4 |
| SL | WT | 100 | 12 | 9 | A2 | 104 | 104 |
| SL | WT | 50 | 12 | 5 | A2 | 60 | 60 |
| SL | WT | 0 | 12 | 1 | C3 | 111 | 5 |
| SL | BI | 0 | 12 | 1 | C3 | 147 | 8 |
| SL | BI | 50 | 12 | 3 | C2 | 109 | 107 |
| SL | BI | 0 | 12 | 8 | B2 | 134 | 6 |
| SL | BI | 50 | 12 | 3 | B2 | 103 | 103 |
| SL | BI | 100 | 12 | 4 | B3 | 126 | 124 |
| SL | BI | 0 | 12 | 1 | C2 | 111 | 3 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SL | BI | 100 | 12 | 5 | B1 | 105 | 105 |
| SL | BI | 50 | 12 | 7 | B1 | 128 | 124 |
| SL | BI | 0 | 12 | 8 | B3 | 163 | 4 |
| SL | BI | 100 | 12 | 4 | B2 | 123 | 122 |
| SL | BI | 100 | 12 | 4 | C3 | 104 | 104 |
| SL | BI | 50 | 12 | 7 | B3 | 109 | 103 |
| SL | BI | 50 | 12 | 7 | B2 | 116 | 113 |
| SL | BI | 0 | 12 | 8 | B1 | 81 | 5 |
| SL | BI | 100 | 12 | 5 | B2 | 97 | 97 |
| SL | BI | 50 | 12 | 7 | A2 | 115 | 113 |
| SL | BI | 100 | 12 | 6 | B3 | 113 | 113 |
| SL | BI | 0 | 12 | 8 | A3 | 140 | 7 |
| SL | BI | 0 | 12 | 9 | B2 | 87 | 2 |
| SL | BI | 50 | 12 | 2 | A2 | 119 | 115 |
| SL | BI | 50 | 12 | 2 | B1 | 105 | 102 |
| SL | BI | 100 | 12 | 6 | B2 | 121 | 119 |
| SL | BI | 100 | 12 | 6 | C2 | 127 | 126 |
| SL | BI | 100 | 12 | 4 | A2 | 117 | 116 |
| SL | BI | 50 | 12 | 3 | B1 | 144 | 136 |
| SL | BI | 100 | 12 | 5 | B3 | 108 | 108 |
| SL | BI | 100 | 12 | 6 | C3 | 98 | 95 |
| SL | BI | 0 | 12 | 1 | A3 | 107 | 7 |
| SL | BI | 100 | 12 | 5 | A2 | 106 | 104 |
| SL | BI | 0 | 12 | 9 | B3 | 137 | 10 |
| SL | BI | 50 | 12 | 2 | B2 | 116 | 114 |
| SL | BI | 0 | 12 | 9 | B1 | 84 | 4 |
| SL | BI | 0 | 12 | 9 | A2 | 115 | 0 |
| SL | BI | 50 | 12 | 3 | A2 | 117 | 113 |
| SL | BI | 0 | 12 | 1 | C1 | 137 | 10 |
| SL | BI | 50 | 12 | 2 | A3 | 145 | 140 |

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# Appendix 9. Statistical Analysis including SAS Programs, Outputs and Logs for Survival Data

| Item Number | Item Description | Number of Pages | Report Page Number |
|---|---|---|---|
| 1 | SAS program for zebra mussel survival data | 7 | 452 |
| 2 | SAS log for zebra mussel survival data | 13 | 459 |
| 3 | SAS output for zebra mussel survival data | 39 | 472 |

```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Statistical analysis of zebra mussel mortality after';
title2 h=1 'exposure to various concentrations of Pf-CL145A';
title3 h=1 'SAS v. 9.3  Analysis completion date:  01MAY2014  Analysis prepare
title4 h=1 ' ';
/*****************************************************
* SAS ver 9.3       Analysis prepared by:  KLW klw    *
* Analysis completion date:  01MAY2014       1MAY2014 *
*****************************************************/

/*****************************************************************************
* Variable Names:
* loc = test location LC=Lake Carlos; SL = Shawano Lake
* trt_typ = exposure method WT = whole water application; BI = bottom injectic
* conc = concentration (in mg/L)
*             50 = 50 mg/L active ingredient
*             100 = 100 mg/L active ingredient
* tnk = test tank ID (1 to 9)
* time = time post exposure assessment occurred
* tray = zebra mussel tray within tank - there were three mussel trays at eact
* tot = total number of zebra mussels at risk
* dead = number of zebra mussels dead after treatment
*****************************************************************************

data mussel; set Pseudo04.survivaldata;
pctsurv = (tot-dead)/tot*100;
pctmort = dead/tot*100;
if conc = 0 then conca = 'C';
if conc = 50 then conca = 'A';
if conc = 100 then conca = 'B';
run;

proc sort data=mussel; by loc trt_typ conc time; run;
proc print data=mussel; title4 h=1 'all data'; run;
Title1 h=2 'The mean percent survival and mortality by location and treatment
title2 h=2 ' classified by concentration and exposure duration';
proc means data = mussel mean std lclm uclm fw=8;
by loc trt_typ;
class conc time;
var pctsurv pctmort;
run;
```

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Item No. 2
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```
data Carlos_6; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
if time ne 6 then delete;
run;
data Carlos_9; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
if time ne 9 then delete;
run;
data Carlos_12; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
if time ne 12 then delete;
run;

data Shawano_6; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
if time ne 6 then delete;
run;
data Shawano_9; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
if time ne 9 then delete;
run;
data Shawano_12; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
if time ne 12 then delete;
run;

data time_Carlos; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
run;

data time_Shawano; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
run;

data BI_Carlos; set mussel;
if trt_typ = 'WT' then delete;
if loc = 'SL' then delete;
data BI_Shawano; set mussel;
```

```
if trt_typ = 'WT' then delete;
if loc = 'LC' then delete;

data BI_vs_WT_Carlos; set mussel;
if time < 12 then delete;
if loc = 'SL' then delete;
run;
data BI_vs_WT_Shawano; set mussel;
if time < 12 then delete;
if loc = 'LC' then delete;
run;

/*************************-----Remarks-----*********************************
* This analysis compares the effect of exposure concentration on zebra mussel
* survival for 6 h WT (whole water treatment)at Lake Carlos only.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration on zet
survival for 6 h WT at Lake Carlos';
proc glimmix data = Carlos_6;
title4  Zebra mussel mortality - 6 h at Lake Carlos only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----*********************************
* This analysis compares the effect of exposure concentration on zebra mussel
* survival for 9 h WT (whole water treatment)at Lake Carlos only.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration on zet
survival for 9 h WT at Lake Carlos';
proc glimmix data = Carlos_9;
title4 'Zebra mussel mortality - 9 h at Lake Carlos only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----*********************************
* This analysis compares the effect of exposure concentration on zebra mussel
* survival for 12 h WT (whole water treatment)at Lake Carlos only.
****************************************************************************
title1 h=2 This analysis looks at the effect of exposure concentration on zet
survival for 12 h WT at Lake Carlos';
```

```
proc glimmix data = Carlos_12;
title4 'Zebra mussel mortality - 12 h at Lake Carlos only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----**********************************
* This analysis compares the effect of exposure concentration on zebra mussel
* survival for 6 h WT (whole water treatment)at Shawano Lake only.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration on zeb
survival for 6 h WT at Shawaro Lake';
proc glimmix data = Shawano_6;
title4 'Zebra mussel mortality - 6 h at Shawano Lake only';
class conca;
model dead/tot = conca / d = bin link = logit roint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----**********************************
* This analysis compares the effect of exposure concentration on zebra mussel
* survival for 9 h WT (whole water treatment)at Shawano Lake only.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration on zeb
survival for 9 h WT at Shawano Lake';
proc glimmix data = Shawano_9;
title4 'Zebra mussel mortality - 9 h at Shawano Lake only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----**********************************
* This analysis compares the effect of exposure concentration on zebra mussel
* survival for 12 h WT (whole water treatment)at Shawano Lake only.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration on zeb
survival for 12 h WT at Shawano Lake';
proc glimmix data = Shawano_12;
title4 'Zebra mussel mortality - 12 h at Shawano Lake only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
```

```
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/**************************-----Remarks-----*********************************
* This analysis compares the effect of exposure duration on zebra mussel
* survival for the WT (whole water treatment)at Lake Carlos only. Note: BI onl
* 12-h exposure period and was therefore excluded from the analysis set. The
* full data set was reduced to contain all observations for WT at LC.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure duration at Lake Car
title2 h=2 'Therefore BI data were excluded from this analysis';
title3 h=2 'Includes 6, 9 and 12 h WWC Lake Carlos data';
proc glimmix data = time_Carlos;
title4 'Zebra mussel mortality - time at Lake Carlos only';
class time conca;
model dead/tot = time|conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans time /pdiff cl ilink or;
  lsmeans conca*time /pdiff cl ilink or;
  random _residual_;
  run;

/**************************-----Remarks-----*********************************
* This analysis compares the effect of exposure duration on zebra mussel
* survival for the WT (whole water treatment)at Shawano Lake only. Note: BI or
* 12-h exposure period and was therefore excluded from the analysis set. The
* full data set was reduced to contain all observations for WT at SL.
****************************************************************************
title1 h=2 'This analysis looks at the effect of exposure duration at Shawano
title2 h=2 'Therefore BI data were excluded from this analysis';
title3 h=2 'Includes 6, 9 and 12 h WWC Shawano Lake data';
proc glimmix data = time_Shawano;
title4 'Zebra mussel mortality - time at Shawano Lake only';
class time conca;
model dead/tot = time|conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans time /pdiff cl ilink or;
  lsmeans conca*time /pdiff cl ilink or;
  random _residual_;
  run;

/**************************-----Remarks-----*********************************
* This analysis compares the effect of treatment method BI (bottom injection)
* on zebra mussel survival at Lake Carlos. Note: BI only used a 12-h exposure
* period - thus the full data set was reduced to contain only those observatic
```

```
* for 12-h BI at LC.
*****************************************************************************
proc glimmix data = BI_Carlos;
title1 h=3 'Zebra mussel mortality - BI application';
title2 h=2 'This analysis only looks at 12h BI at Lake Carlos';
class conca;
model dead/tot  = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----**********************************
* This analysis compares the effect of treatment method BI (bottom injection)
* on zebra mussel survival at Shawano Lake. Note: BI only used a 12-h exposure
* period - thus the full data set was reduced to contain only those observatic
* for 12-h BI at SL.
*****************************************************************************
proc glimmix data = BI_Shawano;
title1 h=3 'Zebra mussel mortality - BI application';
title2 h=2 'This analysis only looks at 12h BI at Shawano Lake';
class conca;
model dead/tot  = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;

/*************************-----Remarks-----**********************************
* This analysis compares the effect of treatment method BI (bottom injection)
* vs WT (whole water treatment) on zebra mussel survival at Lake Carlos.
* Note: BI only used a 12-h exposure period - thus the full data set was
* reduced to contain only those observations for 12-h for both BI and WT at LC
*****************************************************************************
proc glimmix data = BI_vs_WT_Carlos;
title1 h=3 'Zebra mussel mortality - application method';
title2 h=2 'This analysis only looks at 12h WT vs 12h BI at Lake Carlos';
title3 h=2 'BI only had 12h exposure so can only compare to 12h WT treatment';
class conca loc trt_typ tnk tray;
model dead/tot  = conca|trt_typ / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans trt_typ /pdiff cl ilink or;
  lsmeans conca*trt_typ /pdiff cl ilink or;
  random _residual_;
  random tnk tray(tnk);
  run;

/*************************-----Remarks-----**********************************
```

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```
* This analysis compares the effect of treatment method BI (bottom injection)
* vs WT (whole water treatment) on zebra mussel survival at Shawano Lake.
* Note: BI only used a 12-h exposure period - thus the full data set was
* reduced to contain only those observations for 12-h for both BI and WT at SL
****************************************************************************
proc glimmix data = BI_vs_WT_Shawano;
title1 h=3 'Zebra mussel mortality - application method';
title2 h=2 'This analysis only looks at 12h WT vs 12h BI at Shawano Lake';
title3 h=2 'BI only had 12h exposure so can only compare to 12h WT treatment';
class conca loc trt_typ tnk tray;
model dead/tot  = conca|trt_typ / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans trt_typ /pdiff cl ilink or;
  lsmeans conca*trt_typ /pdiff cl ilink or;
  random _residual_;
  random tnk tray(tnk);
  run;
```

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```
DM 'LOG; CLEAR; OUTPUT; CLEAR;';  * CLEAR LOG AND OUTPUT;

FOOTNOTE1 'Performed by K. Weber SAS version ' &SYSVER &SYSTIME &SYSDATE;
WARNING: The FOOTNOTE statement is ambiguous due to invalid options or
         unquoted text.

options ls=97 ps=54 formdlim='-' pageno = 1 nocenter nodate nosource2;

title1 h=1 'Statistical analysis of zebra mussel mortality after';
title2 h=1 'exposure to various concentrations of Pf-CL145A';
title3 h=1 'SAS v. 9.3  Analysis completion date:  01MAY2014  Analysis pr
title4 h=1 ' ';
/*****************************************************
* SAS ver 9.3       Analysis prepared by:  KLW KLW        *
* Analysis completion date:  01MAY2014          1MAY2014 *
*****************************************************/

/************************************************************************
 *************
* Variable Names:
            *
* loc = test location LC=Lake Carlos; SL = Shawano Lake
            *
* trt_typ = exposure method WT = whole water application; BI = bottom inj
            *
* conc = concentration (in mg/L)
            *
*             50 = 50 mg/L active ingredient
            *
*             100 = 100 mg/L active ingredient
            *
* tnk = test tank ID (1 to 9)
            *
* time = time post exposure assessment occurred
            *
* tray = zebra mussel tray within tank - there were three mussel trays at
 each tank  *
* tot = total number of zebra mussels at risk
            *
* dead = number of zebra mussels dead after treatment
            *
************************************************************************
 *************/

data mussel; set Pseudo04.survivaldata;
pctsurv = (tot-dead)/tot*100;
```

FF # 18
Item No. 3
Pg 1 of 13

```
pctmort = dead/tot*100;
if conc = 0 then conca = 'C';
if conc = 50 then conca = 'A ;
if conc = 100 then conca = 'B';
run;

NOTE: There were 225 observations read from the data set PSEUDO04.SURVIVALDATA
NOTE: The data set WORK.MUSSEL has 225 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.10 seconds
      cpu time            0.00 seconds


proc sort data=mussel; by loc trt_typ conc time; run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.MUSSEL has 225 observations and 11 variables.
NOTE: PROCEDURE SORT used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


proc print data=mussel; title4 h=1 'all data'; run;
NOTE: Writing HTML Body file: sashtml.htm
NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE PRINT used (Total process time):
      real time           0.67 seconds
      cpu time            0.49 seconds


Title1 h=2 'The mean percent survival and mortality by location and treat
title2 h=2 ' classified by concentration and exposure duration';
proc means data = mussel mean std lclm uclm fw=8;
by loc trt_typ;
class conc time;
var pctsurv pctmort;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: PROCEDURE MEANS used (Total process time):
      real time           0.20 seconds
      cpu time            0.07 seconds


data Carlos_6; set mussel;
```

```
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
if time ne 6 then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.CARLOS_6 has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data Carlos_9; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
if time ne 9 then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.CARLOS_9 has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data Carlos_12; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
if time ne 12 then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.CARLOS_12 has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data Shawano_6; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
if time ne 6 then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
```

```
NOTE: The data set WORK.SHAWANO_6 has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data Shawano_9; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
if time ne 9 then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.SHAWANO_9 has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data Shawano_12; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
if time ne 12 then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.SHAWANO_12 has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


data time_Carlos; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'SL' then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.TIME_CARLOS has 81 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds
```

```
data time_Shawano; set mussel;
if trt_typ = 'BI' then delete;
if loc = 'LC' then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.TIME_SHAWANO has 81 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data BI_Carlos; set mussel;
if trt_typ = 'WT' then delete;
if loc = 'SL' then delete;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.BI_CARLOS has 27 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data BI_Shawano; set mussel;
if trt_typ = 'WT' then delete;
if loc = 'LC' then delete;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.BI_SHAWANO has 36 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.00 seconds
      cpu time            0.00 seconds


data BI_vs_WT_Carlos; set mussel;
if time < 12 then delete;
if loc = 'SL' then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.BI_VS_WT_CARLOS has 54 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.03 seconds
      cpu time            0.00 seconds
```

```
data BI_vs_WT_Shawano; set mussel;
if time < 12 then delete;
if loc = 'LC' then delete;
run;

NOTE: There were 225 observations read from the data set WORK.MUSSEL.
NOTE: The data set WORK.BI_VS_WT_SHAWANO has 63 observations and 11 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


/**************************-----Remarks-----*****************************
* This analysis compares the effect of exposure concentration on zebra mu
* survival for 6 h WT (whole water treatment)at Lake Carlos only.
*************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration c
survival for 6 h WT at Lake Carlos';
proc glimmix data = Carlos_6;
title4 'Zebra mussel mortality - 6 h at Lake Carlos only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;



NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.26 seconds
      cpu time            0.10 seconds


/**************************-----Remarks-----*****************************
* This analysis compares the effect of exposure concentration on zebra mu
* survival for 9 h WT (whole water treatment)at Lake Carlos only.
*************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration c
survival for 9 h WT at Lake Carlos';
```

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```
proc glimmix data = Carlos_9;
title4 'Zebra mussel mortality - 9 h at Lake Carlos only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;
```

```
NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.21 seconds
      cpu time            0.15 seconds
```

```

/**************************-----Remarks-----*****************************
* This analysis compares the effect of exposure concentration on zebra mu
* survival for 12 h WT (whole water treatment)at Lake Carlos only.
***********************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration c
survival for 12 h WT at Lake Carlos';
proc glimmix data = Carlos_12;
title4 'Zebra mussel mortality - 12 h at Lake Carlos only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;
```

```
NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.23 seconds
      cpu time            0.14 seconds
```

```

/**************************-----Remarks-----*****************************
* This analysis compares the effect of exposure concentration on zebra mu
```

```
* survival for 6 h WT (whole water treatment)at Shawano Lake only.
**************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration c
survival for 6 h WT at Shawano Lake';
proc glimmix data = Shawano_6;
title4 'Zebra mussel mortality - 6 h at Shawano Lake only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;
```

```
NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.29 seconds
      cpu time            0.17 seconds
```

```

/*************************-----Remarks-----*****************************
* This analysis compares the effect of exposure concentration on zebra mu
* survival for 9 h WT (whole water treatment)at Shawano Lake only.
**************************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration c
survival for 9 h WT at Shawano Lake';
proc glimmix data = Shawano_9;
title4 'Zebra mussel mortality - 9 h at Shawano Lake only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;
```

```
NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.37 seconds
      cpu time            0.15 seconds
```

```

/*************************-----Remarks-----****************************
* This analysis compares the effect of exposure concentration on zebra mu
* survival for 12 h WT (whole water treatment)at Shawano Lake only.
***********************************************************************
title1 h=2 'This analysis looks at the effect of exposure concentration c
survival for 12 h WT at Shawano Lake';
proc glimmix data = Shawano_12;
title4 'Zebra mussel mortality - 12 h at Shawano Lake only';
class conca;
model dead/tot = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;



NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.49 seconds
      cpu time            0.10 seconds



/*************************-----Remarks-----****************************
* This analysis compares the effect of exposure duration on zebra mussel
* survival for the WT (whole water treatment)at Lake Carlos only. Note: E
* 12-h exposure period and was therefore excluded from the analysis set.
* full data set was reduced to contain all observations for WT at LC.
***********************************************************************
title1 h=2 'This analysis looks at the effect of exposure duration at Lak
title2 h=2 'Therefore BI data were excluded from this analysis';
title3 h=2 'Includes 6, 9 and 12 h WWC Lake Carlos data';
proc glimmix data = time_Carlos;
title4 'Zebra mussel mortality - time at Lake Carlos only';
class time conca;
model dead/tot = time|conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans time /pdiff cl ilink or;
  lsmeans conca*time /pdiff cl ilink or;
  random _residual_;
  run;
```

```
NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.35 seconds
      cpu time            0.20 seconds


/*************************-----Remarks-----****************************
 * This analysis compares the effect of exposure duration on zebra mussel
 * survival for the WT (whole water treatment)at Shawano Lake only. Note:
 * 12-h exposure period and was therefore excluded from the analysis set.
 * full data set was reduced to contain all observations for WT at SL.
 **********************************************************************
 title1 h=2 'This analysis looks at the effect of exposure duration at Sha
 title2 h=2 'Therefore BI data were excluded from this analysis';
 title3 h=2 'Includes 6, 9 and 12 h WWC Shawano Lake data';
 proc glimmix data = time_Shawano;
 title4 'Zebra mussel mortality - time at Shawano Lake only';
 class time conca;
 model dead/tot = time|conca / d = bin link = logit noint s or;
   lsmeans conca /pdiff cl ilink or;
   lsmeans time /pdiff cl ilink or;
   lsmeans conca*time /pdiff cl ilink or;
   random _residual_;
   run;


NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.35 seconds
      cpu time            0.18 seconds


/*************************-----Remarks-----****************************
 * This analysis compares the effect of treatment method BI (bottom inject
 * on zebra mussel survival at Lake Carlos. Note: BI only used a 12-h expo
 * period - thus the full data set was reduced to contain only those obser
 * for 12-h BI at LC.
```

```
*************************************************************************
proc glimmix data = BI_Carlos;
title1 h=3 'Zebra mussel mortality - BI application';
title2 h=2 'This analysis only looks at 12h BI at Lake Carlos';
class conca;
model dead/tot  = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;


NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (GCONV=1E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.24 seconds
      cpu time            0.14 seconds


/**************************-----Remarks-----*****************************
* This analysis compares the effect of treatment method BI (bottom inject
* or zebra mussel survival at Shawano Lake. Note: BI only used a 12-h exp
* period - thus the full data set was reduced to contain only those obser
* for 12-h BI at SL.
*************************************************************************
proc glimmix data = BI_Shawano;
title1 h=3 'Zebra mussel mortality - BI application';
title2 h=2 'This analysis only looks at 12h BI at Shawano Lake';
class conca;
model dead/tot  = conca / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  random _residual_;
  run;


NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (ABSGCONV=0.00001) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.23 seconds
      cpu time            0.09 seconds
```

```
/**************************-----Remarks-----*****************************
* This analysis compares the effect of treatment method BI (bottom inject
* vs WT (whole water treatment) on zebra mussel survival at Lake Carlos.
* Note: BI only used a 12-h exposure period - thus the full data set was
* reduced to contain only those observations for 12-h for both BI and WT
*************************************************************************
proc glimmix data = BI_vs_WT_Carlos;
title1 h=3 'Zebra mussel mortality - application method';
title2 h=2 'This analysis only looks at 12h WT vs 12h BI at Lake Carlos';
title3 h=2 'BI only had 12h exposure so can only compare to 12h WT treatm
class conca loc trt_typ tnk tray;
model dead/tot  = conca|trt_typ / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans trt_typ /pdiff cl ilink or;
  lsmeans conca*trt_typ /pdiff cl ilink or;
  random _residual_;
  random tnk tray(tnk);
  run;


NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (PCONV=1.11022E-8) satisfied.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.45 seconds
      cpu time            0.23 seconds


/**************************-----Remarks-----*****************************
* This analysis compares the effect of treatment method BI (bottom inject
* vs WT (whole water treatment) on zebra mussel survival at Shawano Lake.
* Note: BI only used a 12-h exposure period - thus the full data set was
* reduced to contain only those observations for 12-h for both BI and WT
*************************************************************************
proc glimmix data = BI_vs_WT_Shawano;
title1 h=3 'Zebra mussel mortality - application method';
title2 h=2 'This analysis only looks at 12h WT vs 12h BI at Shawano Lake'
title3 h=2 'BI only had 12h exposure so can only compare to 12h WT treatm
class conca loc trt_typ tnk tray;
model dead/tot  = conca|trt_typ / d = bin link = logit noint s or;
  lsmeans conca /pdiff cl ilink or;
  lsmeans trt_typ /pdiff cl ilink or;
  lsmeans conca*trt_typ /pdiff cl ilink or;
  random _residual_;
```

```
292     random tnk tray(tnk);
293     run;
```

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[handwritten initials] 1 MAY 2014

```
NOTE: The model does not contain an intercept. Columns of X are scaled only ar
NOTE: Convergence criterion (PCONV=1.11022E-8) satisfied.
NOTE: Estimated G matrix is not positive definite.
NOTE: PROCEDURE GLIMMIX used (Total process time):
      real time           0.37 seconds
      cpu time            0.18 seconds
```

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Item No. 3
Pg 13 of 13

AEM-12-PSEUDO-01

Statistical analysis of zebra mussel mortality after
exposure to various concentrations of Pf-CL145A
SAS v. 9.3 Analysis completion date: 01MAY2014 Analysis prepared by: KLW
all data

KLW 1MAY2014

| Obs | loc | trt_typ | conc | time | tnk | tray | tot | dead | pctsurv | pctmort | conca |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | LC | BI | 0 | 12 | 6 | C1 | 179 | 4 | 97.765 | 2.235 | C |
| 2 | LC | BI | 0 | 12 | 7 | A1 | 225 | 4 | 98.222 | 1.778 | C |
| 3 | LC | BI | 0 | 12 | 3 | C3 | 253 | 7 | 97.233 | 2.767 | C |
| 4 | LC | BI | 0 | 12 | 7 | B3 | 176 | 9 | 94.886 | 5.114 | C |
| 5 | LC | BI | 0 | 12 | 3 | B1 | 242 | 5 | 97.934 | 2.066 | C |
| 6 | LC | BI | 0 | 12 | 6 | B1 | 217 | 11 | 94.931 | 5.069 | C |
| 7 | LC | BI | 0 | 12 | 6 | A2 | 186 | 4 | 97.849 | 2.151 | C |
| 8 | LC | BI | 0 | 12 | 3 | A3 | 196 | 4 | 97.959 | 2.041 | C |
| 9 | LC | BI | 0 | 12 | 7 | C1 | 276 | 3 | 98.913 | 1.087 | C |
| 10 | LC | BI | 50 | 12 | 4 | B2 | 256 | 191 | 25.391 | 74.609 | A |
| 11 | LC | BI | 50 | 12 | 1 | B3 | 279 | 217 | 22.222 | 77.778 | A |
| 12 | LC | BI | 50 | 12 | 8 | C2 | 200 | 161 | 19.500 | 80.500 | A |
| 13 | LC | BI | 50 | 12 | 1 | B2 | 510 | 424 | 16.863 | 83.137 | A |
| 14 | LC | BI | 50 | 12 | 4 | B1 | 188 | 178 | 5.319 | 94.681 | A |
| 15 | LC | BI | 50 | 12 | 4 | B3 | 188 | 174 | 7.447 | 92.553 | A |
| 16 | LC | BI | 50 | 12 | 8 | B3 | 291 | 222 | 23.711 | 76.289 | A |
| 17 | LC | BI | 50 | 12 | 8 | A3 | 282 | 232 | 17.730 | 82.270 | A |
| 18 | LC | BI | 50 | 12 | 1 | A1 | 210 | 158 | 24.762 | 75.238 | A |
| 19 | LC | BI | 100 | 12 | 9 | A1 | 269 | 256 | 4.833 | 95.167 | B |
| 20 | LC | BI | 100 | 12 | 9 | B3 | 179 | 139 | 22.346 | 77.654 | B |
| 21 | LC | BI | 100 | 12 | 2 | B3 | 153 | 132 | 13.725 | 86.275 | B |
| 22 | LC | BI | 100 | 12 | 2 | C1 | 193 | 154 | 20.207 | 79.793 | B |
| 23 | LC | BI | 100 | 12 | 9 | C1 | 288 | 263 | 8.681 | 91.319 | B |
| 24 | LC | BI | 100 | 12 | 5 | A1 | 329 | 217 | 34.043 | 65.957 | B |
| 25 | LC | BI | 100 | 12 | 5 | B3 | 393 | 272 | 30.789 | 69.211 | B |
| 26 | LC | BI | 100 | 12 | 2 | A1 | 279 | 261 | 6.452 | 93.548 | B |
| 27 | LC | BI | 100 | 12 | 5 | C1 | 264 | 209 | 20.833 | 79.167 | B |
| 28 | LC | WT | 0 | 6 | 5 | C1 | 175 | 0 | 100.000 | 0.000 | C |
| 29 | LC | WT | 0 | 6 | 2 | C1 | 168 | 3 | 98.214 | 1.786 | C |
| 30 | LC | WT | 0 | 6 | 2 | B2 | 237 | 10 | 95.781 | 4.219 | C |
| 31 | LC | WT | 0 | 6 | 3 | A1 | 328 | 23 | 92.988 | 7.012 | C |
| 32 | LC | WT | 0 | 6 | 5 | B2 | 173 | 2 | 98.844 | 1.156 | C |
| 33 | LC | WT | 0 | 6 | 3 | B1 | 255 | 3 | 98.824 | 1.176 | C |
| 34 | LC | WT | 0 | 6 | 2 | A1 | 339 | 2 | 99.410 | 0.590 | C |
| 35 | LC | WT | 0 | 6 | 5 | A1 | 269 | 5 | 98.141 | 1.859 | C |
| 36 | LC | WT | 0 | 6 | 3 | C3 | 310 | 6 | 98.065 | 1.935 | C |
| 37 | LC | WT | 0 | 9 | 5 | B3 | 192 | 7 | 96.354 | 3.646 | C |
| 38 | LC | WT | 0 | 9 | 3 | A3 | 178 | 7 | 96.067 | 3.933 | C |
| 39 | LC | WT | 0 | 9 | 5 | C3 | 173 | 11 | 93.642 | 6.358 | C |
| 40 | LC | WT | 0 | 9 | 2 | C3 | 226 | 6 | 97.345 | 2.655 | C |
| 41 | LC | WT | 0 | 9 | 3 | B3 | 213 | 2 | 99.061 | 0.939 | C |
| 42 | LC | WT | 0 | 9 | 3 | C2 | 214 | 11 | 94.860 | 5.140 | C |
| 43 | LC | WT | 0 | 9 | 2 | B3 | 236 | 6 | 97.458 | 2.542 | C |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | LC | WT | 0 | 9 | 5 | A3 | 177 | 2 | 98.870 | 1 130 | C |
| 45 | LC | WT | 0 | 9 | 2 | A3 | 326 | 6 | 98.160 | 1.840 | C |
| 46 | LC | WT | 0 | 12 | 3 | B2 | 312 | 8 | 97.436 | 2.564 | C |
| 47 | LC | WT | 0 | 12 | 3 | A2 | 185 | 5 | 97.297 | 2.703 | C |
| 48 | LC | WT | 0 | 12 | 2 | A2 | 390 | 16 | 95.897 | 4.103 | C |
| 49 | LC | WT | 0 | 12 | 3 | C1 | 259 | 5 | 98.069 | 1.931 | C |
| 50 | LC | WT | 0 | 12 | 2 | B1 | 185 | 4 | 97.838 | 2.162 | C |
| 51 | LC | WT | 0 | 12 | 2 | C2 | 198 | 8 | 95.960 | 4.040 | C |
| 52 | LC | WT | 0 | 12 | 5 | A2 | 240 | 2 | 99.167 | 0.833 | C |
| 53 | LC | WT | 0 | 12 | 5 | C2 | 212 | 6 | 97.170 | 2.830 | C |
| 54 | LC | WT | 0 | 12 | 5 | B1 | 196 | 6 | 96.939 | 3.061 | C |
| 55 | LC | WT | 50 | 6 | 1 | A3 | 149 | 149 | 0.000 | 100.000 | A |
| 56 | LC | WT | 50 | 6 | 4 | C2 | 175 | 171 | 2.286 | 97.714 | A |
| 57 | LC | WT | 50 | 6 | 8 | B1 | 208 | 203 | 2.404 | 97.596 | A |
| 58 | LC | WT | 50 | 6 | 1 | A2 | 280 | 274 | 2.143 | 97.857 | A |
| 59 | LC | WT | 50 | 6 | 8 | B2 | 178 | 177 | 0.562 | 99.438 | A |
| 60 | LC | WT | 50 | 6 | 4 | B2 | 214 | 210 | 1.869 | 98.131 | A |
| 61 | LC | WT | 50 | 6 | 1 | B2 | 232 | 228 | 1.724 | 98.276 | A |
| 62 | LC | WT | 50 | 6 | 4 | C1 | 191 | 191 | 0.000 | 100.000 | A |
| 63 | LC | WT | 50 | 6 | 8 | B3 | 248 | 247 | 0.403 | 99.597 | A |
| 64 | LC | WT | 50 | 9 | 4 | B3 | 265 | 261 | 1.509 | 98.491 | A |
| 65 | LC | WT | 50 | 9 | 4 | A3 | 375 | 375 | 0.000 | 100.000 | A |
| 66 | LC | WT | 50 | 9 | 8 | C1 | 169 | 169 | 0.000 | 100.000 | A |
| 67 | LC | WT | 50 | 9 | 1 | A1 | 164 | 161 | 1.829 | 98.171 | A |
| 68 | LC | WT | 50 | 9 | 4 | A1 | 221 | 221 | 0.000 | 100.000 | A |
| 69 | LC | WT | 50 | 9 | 8 | C3 | 180 | 180 | 0.000 | 100.000 | A |
| 70 | LC | WT | 50 | 9 | 8 | A3 | 131 | 127 | 3.053 | 96.947 | A |
| 71 | LC | WT | 50 | 9 | 1 | B1 | 128 | 123 | 3.906 | 96.094 | A |
| 72 | LC | WT | 50 | 9 | 1 | C1 | 267 | 267 | 0.000 | 100.000 | A |
| 73 | LC | WT | 50 | 12 | 1 | C3 | 266 | 266 | 0.000 | 100.000 | A |
| 74 | LC | WT | 50 | 12 | 1 | C2 | 175 | 175 | 0.000 | 100.000 | A |
| 75 | LC | WT | 50 | 12 | 1 | B3 | 160 | 159 | 0.625 | 99.375 | A |
| 76 | LC | WT | 50 | 12 | 8 | C2 | 290 | 287 | 1.034 | 98.966 | A |
| 77 | LC | WT | 50 | 12 | 8 | A2 | 119 | 119 | 0.000 | 100.000 | A |
| 78 | LC | WT | 50 | 12 | 8 | A1 | 151 | 150 | 0.662 | 99.338 | A |
| 79 | LC | WT | 50 | 12 | 4 | A2 | 158 | 155 | 1.899 | 98.101 | A |
| 80 | LC | WT | 50 | 12 | 4 | C3 | 455 | 453 | 0.440 | 99.560 | A |
| 81 | LC | WT | 50 | 12 | 4 | B1 | 280 | 277 | 1.071 | 98.929 | A |
| 82 | LC | WT | 100 | 6 | 7 | B2 | 266 | 256 | 3.759 | 96.241 | B |
| 83 | LC | WT | 100 | 6 | 9 | B2 | 190 | 188 | 1.053 | 98.947 | B |
| 84 | LC | WT | 100 | 6 | 6 | C3 | 267 | 264 | 1.124 | 98.876 | B |
| 85 | LC | WT | 100 | 6 | 7 | A1 | 212 | 210 | 0.943 | 99.057 | B |
| 86 | LC | WT | 100 | 6 | 6 | A3 | 235 | 227 | 3.404 | 96.596 | B |
| 87 | LC | WT | 100 | 6 | 6 | A2 | 257 | 250 | 2.724 | 97.276 | B |
| 88 | LC | WT | 100 | 6 | 9 | C1 | 324 | 317 | 2.160 | 97.840 | B |
| 89 | LC | WT | 100 | 6 | 7 | C1 | 269 | 267 | 0.743 | 99.257 | B |
| 90 | | | | | | | | | | | |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | LC | WT | 100 | 6 | 9 | A1 | 283 | 275 | 2.827 | 97.173 | B |
| 91 | LC | WT | 100 | 9 | 6 | B2 | 188 | 187 | 0.532 | 99.468 | B |
| 92 | LC | WT | 100 | 9 | 7 | B3 | 193 | 193 | 0.000 | 100.000 | B |
| 93 | LC | WT | 100 | 9 | 7 | C3 | 158 | 155 | 1.899 | 98.101 | B |
| 94 | LC | WT | 100 | 9 | 6 | C1 | 176 | 176 | 0.000 | 100.000 | B |
| 95 | LC | WT | 100 | 9 | 8 | A3 | 393 | 392 | 0.254 | 99.746 | B |
| 96 | LC | WT | 100 | 9 | 9 | B3 | 128 | 128 | 0.000 | 100.000 | B |
| 97 | LC | WT | 100 | 9 | 6 | B3 | 183 | 181 | 1.093 | 98.907 | B |
| 98 | LC | WT | 100 | 9 | 9 | C3 | 260 | 260 | 0.000 | 100.000 | B |
| 99 | LC | WT | 100 | 9 | 7 | A3 | 258 | 255 | 1.163 | 98.837 | B |
| 100 | LC | WT | 100 | 12 | 6 | B1 | 249 | 248 | 0.402 | 99.598 | B |
| 101 | LC | WT | 100 | 12 | 6 | A1 | 239 | 236 | 1.255 | 98.745 | B |
| 102 | LC | WT | 100 | 12 | 9 | A2 | 316 | 316 | 0.000 | 100.000 | B |
| 103 | LC | WT | 100 | 12 | 7 | A2 | 237 | 236 | 0.422 | 99.578 | B |
| 104 | LC | WT | 100 | 12 | 9 | B1 | 273 | 268 | 1.832 | 98.168 | B |
| 105 | LC | WT | 100 | 12 | 7 | B1 | 286 | 286 | 0.000 | 100.000 | B |
| 106 | LC | WT | 100 | 12 | 7 | C2 | 203 | 203 | 0.000 | 100.000 | B |
| 107 | LC | WT | 100 | 12 | 9 | C2 | 156 | 155 | 0.641 | 99.359 | B |
| 108 | LC | WT | 100 | 12 | 6 | C2 | 155 | 153 | 1.290 | 98.710 | B |
| 109 | SL | BI | 0 | 12 | 1 | C3 | 147 | 8 | 94.558 | 5.442 | C |
| 110 | SL | BI | 0 | 12 | 8 | B2 | 134 | 6 | 95.522 | 4.478 | C |
| 111 | SL | BI | 0 | 12 | 1 | C2 | 111 | 3 | 97.297 | 2.703 | C |
| 112 | SL | BI | 0 | 12 | 8 | B3 | 163 | 4 | 97.546 | 2.454 | C |
| 113 | SL | BI | 0 | 12 | 8 | B1 | 81 | 5 | 93.827 | 6.173 | C |
| 114 | SL | BI | 0 | 12 | 8 | A3 | 140 | 7 | 95.000 | 5.000 | C |
| 115 | SL | BI | 0 | 12 | 9 | B2 | 87 | 2 | 97.701 | 2.299 | C |
| 116 | SL | BI | 0 | 12 | 1 | A3 | 107 | 7 | 93.458 | 6.542 | C |
| 117 | SL | BI | 0 | 12 | 9 | B3 | 137 | 10 | 92.701 | 7.299 | C |
| 118 | SL | BI | 0 | 12 | 9 | B1 | 84 | 4 | 95.238 | 4.762 | C |
| 119 | SL | BI | 0 | 12 | 9 | A2 | 115 | 0 | 100.000 | 0.000 | C |
| 120 | SL | BI | 0 | 12 | 1 | C1 | 137 | 10 | 92.701 | 7.299 | C |
| 121 | SL | BI | 50 | 12 | 3 | C2 | 109 | 107 | 1.835 | 98.165 | A |
| 122 | SL | BI | 50 | 12 | 3 | B2 | 103 | 103 | 0.000 | 100.000 | A |
| 123 | SL | BI | 50 | 12 | 7 | B1 | 128 | 124 | 3.125 | 96.875 | A |
| 124 | SL | BI | 50 | 12 | 7 | B3 | 109 | 103 | 5.505 | 94.495 | A |
| 125 | SL | BI | 50 | 12 | 7 | B2 | 116 | 113 | 2.586 | 97.414 | A |
| 126 | SL | BI | 50 | 12 | 7 | A2 | 115 | 113 | 1.739 | 98.231 | A |
| 127 | SL | BI | 50 | 12 | 2 | A2 | 119 | 115 | 3.361 | 96.639 | A |
| 128 | SL | BI | 50 | 12 | 2 | B1 | 105 | 102 | 2.857 | 97.143 | A |
| 129 | SL | BI | 50 | 12 | 3 | B1 | 144 | 136 | 5.556 | 94.444 | A |
| 130 | SL | BI | 50 | 12 | 2 | B2 | 116 | 114 | 1.724 | 98.276 | A |
| 131 | SL | BI | 50 | 12 | 3 | A2 | 117 | 113 | 3.419 | 96.581 | A |
| 132 | SL | BI | 50 | 12 | 2 | A3 | 145 | 140 | 3.448 | 96.552 | A |
| 133 | SL | BI | 100 | 12 | 4 | B3 | 126 | 124 | 1.587 | 98.413 | B |
| 134 | SL | BI | 100 | 12 | 5 | B1 | 105 | 105 | 0.000 | 100.000 | B |
| 135 | SL | BI | 100 | 12 | 4 | B2 | 123 | 122 | 0.813 | 99.187 | B |
| 136 | | | | | | | | | | | |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | BI | 100 | 12 | 4 | C3 | 104 | 104 | 0.000 | 100.000 | B |
| 137 | SL | BI | 100 | 12 | 5 | B2 | 97 | 97 | 0.000 | 100.000 | B |
| 138 | SL | BI | 100 | 12 | 6 | B3 | 113 | 113 | 0.000 | 100.000 | B |
| 139 | SL | BI | 100 | 12 | 6 | B2 | 121 | 119 | 1.653 | 98.347 | B |
| 140 | SL | BI | 100 | 12 | 6 | C2 | 127 | 126 | 0.787 | 99.213 | B |
| 141 | SL | BI | 100 | 12 | 4 | A2 | 117 | 116 | 0.855 | 99.145 | B |
| 142 | SL | BI | 100 | 12 | 5 | B3 | 108 | 108 | 0.000 | 100.000 | B |
| 143 | SL | BI | 100 | 12 | 6 | C3 | 98 | 95 | 3.061 | 96.939 | B |
| 144 | SL | BI | 100 | 12 | 5 | A2 | 106 | 104 | 1.887 | 98.113 | B |
| 145 | SL | WT | 0 | 6 | 7 | B1 | 80 | 2 | 97.500 | 2.500 | C |
| 146 | SL | WT | 0 | 6 | 1 | A1 | 109 | 4 | 96.330 | 3.670 | C |
| 147 | SL | WT | 0 | 6 | 1 | B2 | 87 | 2 | 97.701 | 2.299 | C |
| 148 | SL | WT | 0 | 6 | 7 | C1 | 108 | 5 | 95.370 | 4.630 | C |
| 149 | SL | WT | 0 | 6 | 1 | B1 | 118 | 7 | 94.068 | 5.932 | C |
| 150 | SL | WT | 0 | 6 | 4 | A1 | 64 | 3 | 95.313 | 4.688 | C |
| 151 | SL | WT | 0 | 6 | 7 | A1 | 124 | 2 | 98.387 | 1.613 | C |
| 152 | SL | WT | 0 | 6 | 4 | B1 | 78 | 4 | 94.872 | 5.128 | C |
| 153 | SL | WT | 0 | 6 | 4 | A2 | 74 | 7 | 90.541 | 9.459 | C |
| 154 | SL | WT | 0 | 9 | 4 | C1 | 71 | 2 | 97.183 | 2.817 | C |
| 155 | SL | WT | 0 | 9 | 1 | B3 | 84 | 2 | 97.619 | 2.381 | C |
| 156 | SL | WT | 0 | 9 | 1 | A3 | 159 | 9 | 94.340 | 5.660 | C |
| 157 | SL | WT | 0 | 9 | 7 | B2 | 108 | 6 | 94.444 | 5.556 | C |
| 158 | SL | WT | 0 | 9 | 7 | A3 | 54 | 2 | 96.296 | 3.704 | C |
| 159 | SL | WT | 0 | 9 | 7 | C3 | 111 | 6 | 94.595 | 5.405 | C |
| 160 | SL | WT | 0 | 9 | 4 | B2 | 75 | 3 | 96.000 | 4.000 | C |
| 161 | SL | WT | 0 | 9 | 4 | A3 | 110 | 7 | 93.636 | 6.364 | C |
| 162 | SL | WT | 0 | 9 | 1 | C1 | 144 | 6 | 95.833 | 4.167 | C |
| 163 | SL | WT | 0 | 12 | 1 | A2 | 101 | 5 | 95.050 | 4.950 | C |
| 164 | SL | WT | 0 | 12 | 1 | C2 | 108 | 6 | 94.444 | 5.556 | C |
| 165 | SL | WT | 0 | 12 | 7 | A2 | 92 | 2 | 97.826 | 2.174 | C |
| 166 | SL | WT | 0 | 12 | 4 | C3 | 141 | 4 | 97.163 | 2.837 | C |
| 167 | SL | WT | 0 | 12 | 7 | C2 | 90 | 3 | 96.667 | 3.333 | C |
| 168 | SL | WT | 0 | 12 | 7 | B3 | 104 | 2 | 98.077 | 1.923 | C |
| 169 | SL | WT | 0 | 12 | 4 | B3 | 79 | 3 | 96.203 | 3.797 | C |
| 170 | SL | WT | 0 | 12 | 4 | C2 | 81 | 4 | 95.062 | 4.938 | C |
| 171 | SL | WT | 0 | 12 | 1 | C3 | 111 | 5 | 95.495 | 4.505 | C |
| 172 | SL | WT | 50 | 6 | 8 | C1 | 136 | 133 | 2.206 | 97.794 | A |
| 173 | SL | WT | 50 | 6 | 8 | A3 | 96 | 91 | 5.208 | 94.792 | A |
| 174 | SL | WT | 50 | 6 | 2 | A1 | 108 | 91 | 15.741 | 84.259 | A |
| 175 | SL | WT | 50 | 6 | 5 | B1 | 67 | 59 | 11.940 | 88.060 | A |
| 176 | SL | WT | 50 | 6 | 2 | B1 | 116 | 106 | 8.621 | 91.379 | A |
| 177 | SL | WT | 50 | 6 | 5 | C1 | 85 | 71 | 16.471 | 83.529 | A |
| 178 | SL | WT | 50 | 6 | 2 | C1 | 61 | 57 | 6.557 | 93.443 | A |
| 179 | SL | WT | 50 | 6 | 5 | A1 | 95 | 87 | 8.421 | 91.579 | A |
| 180 | SL | WT | 50 | 6 | 8 | B3 | 149 | 92 | 38.255 | 61.745 | A |
| 181 | SL | WT | 50 | 9 | 8 | C2 | 61 | 60 | 1.639 | 98.361 | A |
| 182 | | | | | | | | | | | |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SL | WT | 50 | 9 | 8 | A1 | 148 | 146 | 1.351 | 98.649 | A |
| 183 | SL | WT | 50 | 9 | 2 | C3 | 117 | 112 | 4.274 | 95.726 | A |
| 184 | SL | WT | 50 | 9 | 8 | B1 | 85 | 80 | 5.882 | 94.118 | A |
| 185 | SL | WT | 50 | 9 | 5 | A3 | 61 | 59 | 3.279 | 96.721 | A |
| 186 | SL | WT | 50 | 9 | 2 | B2 | 103 | 94 | 8.738 | 91.262 | A |
| 187 | SL | WT | 50 | 9 | 5 | C3 | 80 | 61 | 23.750 | 76.250 | A |
| 188 | SL | WT | 50 | 9 | 2 | A3 | 114 | 88 | 22.807 | 77.193 | A |
| 189 | SL | WT | 50 | 9 | 5 | B2 | 105 | 83 | 20.952 | 79.048 | A |
| 190 | SL | WT | 50 | 12 | 5 | C2 | 114 | 112 | 1.754 | 98.246 | A |
| 191 | SL | WT | 50 | 12 | 2 | A2 | 98 | 97 | 1.020 | 98.980 | A |
| 192 | SL | WT | 50 | 12 | 8 | B2 | 88 | 85 | 3.409 | 96.591 | A |
| 193 | SL | WT | 50 | 12 | 2 | C2 | 86 | 86 | 0.000 | 100.000 | A |
| 194 | SL | WT | 50 | 12 | 8 | C3 | 129 | 128 | 0.775 | 99.225 | A |
| 195 | SL | WT | 50 | 12 | 2 | B3 | 85 | 80 | 5.882 | 94.118 | A |
| 196 | SL | WT | 50 | 12 | 5 | B3 | 63 | 58 | 7.937 | 92.063 | A |
| 197 | SL | WT | 50 | 12 | 8 | A2 | 76 | 73 | 3.947 | 96.053 | A |
| 198 | SL | WT | 50 | 12 | 5 | A2 | 60 | 60 | 0.000 | 100.000 | A |
| 199 | SL | WT | 100 | 6 | 9 | A1 | 77 | 75 | 2.597 | 97.403 | B |
| 200 | SL | WT | 100 | 6 | 6 | A3 | 57 | 54 | 5.263 | 94.737 | B |
| 201 | SL | WT | 100 | 6 | 9 | C1 | 118 | 99 | 16.102 | 83.898 | B |
| 202 | SL | WT | 100 | 6 | 3 | B3 | 104 | 95 | 8.654 | 91.346 | B |
| 203 | SL | WT | 100 | 6 | 3 | B2 | 122 | 114 | 6.557 | 93.443 | B |
| 204 | SL | WT | 100 | 6 | 6 | A2 | 124 | 121 | 2.419 | 97.581 | B |
| 205 | SL | WT | 100 | 6 | 3 | A1 | 138 | 111 | 19.565 | 80.435 | B |
| 206 | SL | WT | 100 | 6 | 9 | B1 | 119 | 115 | 3.361 | 96.639 | B |
| 207 | SL | WT | 100 | 6 | 6 | A1 | 69 | 69 | 0.000 | 100.000 | B |
| 208 | SL | WT | 100 | 9 | 3 | B1 | 51 | 49 | 3.922 | 96.078 | B |
| 209 | SL | WT | 100 | 9 | 3 | C2 | 73 | 69 | 5.479 | 94.521 | B |
| 210 | SL | WT | 100 | 9 | 9 | C3 | 123 | 116 | 5.691 | 94.309 | B |
| 211 | SL | WT | 100 | 9 | 3 | C3 | 88 | 88 | 0.000 | 100.000 | B |
| 212 | SL | WT | 100 | 9 | 9 | A3 | 107 | 93 | 13.084 | 86.916 | B |
| 213 | SL | WT | 100 | 9 | 6 | B1 | 59 | 59 | 0.000 | 100.000 | B |
| 214 | SL | WT | 100 | 9 | 9 | B2 | 69 | 69 | 0.000 | 100.000 | B |
| 215 | SL | WT | 100 | 9 | 6 | C2 | 94 | 94 | 0.000 | 100.000 | B |
| 216 | SL | WT | 100 | 9 | 6 | B2 | 94 | 81 | 13.830 | 86.170 | B |
| 217 | SL | WT | 100 | 12 | 6 | C1 | 62 | 59 | 4.839 | 95.161 | B |
| 218 | SL | WT | 100 | 12 | 3 | A3 | 131 | 125 | 4.580 | 95.420 | B |
| 219 | SL | WT | 100 | 12 | 3 | A2 | 95 | 94 | 1.053 | 98.947 | B |
| 220 | SL | WT | 100 | 12 | 9 | C2 | 60 | 58 | 3.333 | 96.667 | B |
| 221 | SL | WT | 100 | 12 | 6 | B3 | 77 | 77 | 0.000 | 100.000 | B |
| 222 | SL | WT | 100 | 12 | 6 | C3 | 102 | 101 | 0.980 | 99.020 | B |
| 223 | SL | WT | 100 | 12 | 3 | C1 | 104 | 103 | 0.962 | 99.038 | B |
| 224 | SL | WT | 100 | 12 | 9 | B3 | 102 | 100 | 1.961 | 98.039 | B |
| 225 | SL | WT | 100 | 12 | 9 | A2 | 104 | 104 | 0.000 | 100.000 | B |

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The mean percent survival and mortality by location and treatment type classified by concentration and exposure duration

AEM-12-PSEUDO-0

The MEANS Procedure

loc=LC trt_typ=BI

| conc | time | N Obs | Variable | Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|---|---|---|---|
| 0 | 12 | 9 | pctsurv | 97.2993 | 1.4252 | 96.2038 | 98.3948 |
| | | | pctmort | 2.7007 | 1.4252 | 1.6052 | 3.7962 |
| 50 | 12 | 9 | pctsurv | 18.1050 | 7.3014 | 12.4927 | 23.7174 |
| | | | pctmort | 81.8950 | 7.3014 | 76.2826 | 87.5073 |
| 100 | 12 | 9 | pctsurv | 17.9899 | 10.3957 | 9.9990 | 25.9807 |
| | | | pctmort | 82.0101 | 10.3957 | 74.0193 | 90.0010 |

loc=LC trt_typ=WT

| conc | time | N Obs | Variable | Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|---|---|---|---|
| 0 | 6 | 9 | pctsurv | 97.8073 | 2.1553 | 96.1506 | 99.4640 |
| | | | pctmort | 2.1927 | 2.1553 | 0.5360 | 3.8494 |
| | 9 | 9 | pctsurv | 96.8685 | 1.8169 | 95.4719 | 98.2651 |
| | | | pctmort | 3.1315 | 1.8169 | 1.7349 | 4.6281 |
| | 12 | 9 | pctsurv | 97.3081 | 1.0183 | 96.5254 | 98.0908 |
| | | | pctmort | 2.6919 | 1.0183 | 1.9092 | 3.4746 |
| 50 | 6 | 9 | pctsurv | 1.2656 | 1.0077 | 0.4911 | 2.0402 |
| | | | pctmort | 98.7344 | 1.0077 | 97.9598 | 99.5089 |
| | 9 | 9 | pctsurv | 1.1443 | 1.5175 | -0.0222 | 2.3107 |
| | | | pctmort | 98.8557 | 1.5175 | 97.6893 | 100.0 |
| | 12 | 9 | pctsurv | 0.6368 | 0.6320 | 0.1510 | 1.1227 |
| | | | pctmort | 99.3632 | 0.6320 | 98.8773 | 99.8490 |
| 100 | 6 | 9 | pctsurv | 2.0820 | 1.1511 | 1.1971 | 2.9668 |
| | | | pctmort | 97.9180 | 1.1511 | 97.0332 | 98.8029 |
| | 9 | 9 | pctsurv | 0.5490 | 0.6880 | 0.0201 | 1.0778 |
| | | | pctmort | 99.4510 | 0.6880 | 98.9222 | 99.9799 |
| | 12 | 9 | pctsurv | 0.6491 | 0.6665 | 0.1368 | 1.1614 |
| | | | pctmort | 99.3509 | 0.6665 | 98.8386 | 99.8632 |

loc=SL trt_typ=BI

| conc | time | N Obs | Variable | Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|---|---|---|---|
| 0 | 12 | 12 | pctsurv | 95.4624 | 2.2632 | 94.0245 | 96.9004 |
| | | | pctmort | 4.5376 | 2.2632 | 3.0996 | 5.9755 |
| 50 | 12 | 12 | pctsurv | 2.9296 | 1.5677 | 1.9335 | 3.9256 |
| | | | pctmort | 97.0704 | 1.5677 | 96.0744 | 98.0665 |
| 100 | 12 | 12 | pctsurv | 0.8869 | 0.9888 | 0.2587 | 1.5152 |
| | | | pctmort | 99.1131 | 0.9888 | 98.4848 | 99.7413 |

loc=SL trt_typ=WT

| conc | time | N Obs | Variable | Mean | Std Dev | Lower 95% CL for Mean | Upper 95% CL for Mean |
|---|---|---|---|---|---|---|---|
| 0 | 6 | 9 | pctsurv | 95.5646 | 2.3698 | 93.7431 | 97.3862 |
| | | | pctmort | 4.4354 | 2.3698 | 2.6138 | 6.2569 |
| | 9 | 9 | pctsurv | 95.5496 | 1.3716 | 94.4954 | 96.6039 |
| | | | pctmort | 4.4504 | 1.3716 | 3.3961 | 5.5046 |
| | 12 | 9 | pctsurv | 96.2207 | 1.2993 | 95.2220 | 97.2195 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | pctmort | 3.7793 | 1.2993 | 2.7805 | 4.7780 |
| 50 | 6 | 9 | pctsurv | 12.6022 | 10.7002 | 4.3773 | 20.8271 |
| | | | pctmort | 87.3978 | 10.7002 | 79.1729 | 95.6227 |
| | 9 | 9 | pctsurv | 10.2969 | 9.4436 | 3.0379 | 17.5560 |
| | | | pctmort | 89.7031 | 9.4436 | 82.4440 | 96.9621 |
| | 12 | 9 | pctsurv | 2.7473 | 2.7746 | 0.6145 | 4.8800 |
| | | | pctmort | 97.2527 | 2.7746 | 95.1200 | 99.3855 |
| 100 | 6 | 9 | pctsurv | 7.1688 | 6.6051 | 2.0917 | 12.2460 |
| | | | pctmort | 92.8312 | 6.6051 | 87.7540 | 97.9083 |
| | 9 | 9 | pctsurv | 4.6673 | 5.5249 | 0.4205 | 8.9141 |
| | | | pctmort | 95.3327 | 5.5249 | 91.0859 | 99.5795 |
| | 12 | 9 | pctsurv | 1.9675 | 1.8541 | 0.5423 | 3.3927 |
| | | | pctmort | 98.0325 | 1.8541 | 96.6073 | 99.4577 |

Performed by K. Weber SAS version 9.3 13:52 01MAY14

This analysis looks at the effect of exposure concentration on zebra musselsurvival for 6 h WT at Lake Carlos

AEH-12-PSEUDO-03

## Zebra mussel mortality - 6 h at Lake Carlos only

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.CARLOS_6 |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 4158 |
| Number of Trials | 6432 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 78.995175605 | . | 7.775484 |
| 1 | 0 | 3 | 75.335487809 | 3.65968760 | 0.906969 |
| 2 | 0 | 3 | 75.284110563 | 0.05137725 | 0.017251 |
| 3 | 0 | 3 | 75.284093161 | 0.00001740 | 6.577E-6 |

Convergence criterion (ABSGCONV=0.00001) satisfied.

AEH-12-PSEUDO-01

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 150.57 |
| AIC (smaller is better) | 156.57 |
| AICC (smaller is better) | 157.61 |
| BIC (smaller is better) | 160.46 |
| CAIC (smaller is better) | 163.46 |
| HQIC (smaller is better) | 157.72 |
| Pearson Chi-Square | 70.70 |
| Pearson Chi-Square / DF | 2.95 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 4.3041 | 0.3456 | 24 | 12.45 | <.0001 |
| conca | B | 3.8286 | 0.2478 | 24 | 15.45 | <.0001 |
| conca | C | -3.7072 | 0.2364 | 24 | -15.68 | <.0001 |
| Resid | | 2.9459 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 213.21 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | >999.999 | 24 | >999.999 | >999.999 |
| B | C | >999.999 | 24 | 924.217 | >999.999 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4.3041 | 0.3456 | 24 | 12.45 | <.0001 | 0.05 | 3.5908 | 5.0173 | 0.9867 | 0.004546 | 0.9732 | 0.9934 |
| B | 3.8286 | 0.2478 | 24 | 15.45 | <.0001 | 0.05 | 3.3171 | 4.3402 | 0.9787 | 0.005161 | 0.9650 | 0.9871 |
| C | -3.7072 | 0.2364 | 24 | -15.68 | <.0001 | 0.05 | -4.1952 | -3.2193 | 0.02396 | 0.005528 | 0.01484 | 0.03845 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | 0.4754 | 0.4253 | 24 | 1.12 | 0.2747 | 0.05 | -0.4023 | 1.3531 | 1.609 | 0.669 | 3.870 |
| A | C | 8.0113 | 0.4187 | 24 | 19.13 | <.0001 | 0.05 | 7.1471 | 8.8755 | >999.999 | >999.999 | >999.999 |
| B | C | 7.5359 | 0.3425 | 24 | 22.00 | <.0001 | 0.05 | 6.8289 | 8.2428 | >999.999 | 924.217 | >999.999 |

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This analysis looks at the effect of exposure concentration on zebra musselsurvival for 9 h WT at Lake Carlos

## Zebra mussel mortality - 9 h at Lake Carlos only

AEH-12-PSEUDO-

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.CARLOS_9 |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 3869 |
| Number of Trials | 5772 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 68.164941544 | . | 6.149662 |
| 1 | 0 | 3 | 62.693908903 | 5.47103264 | 1.263567 |
| 2 | 0 | 3 | 62.408529168 | 0.28537973 | 0.096251 |
| 3 | 0 | 3 | 62.406780661 | 0.00174851 | 0.00069 |
| 4 | 0 | 3 | 62.406780571 | 0.00000009 | 3.608E-8 |

Convergence criterion (GCONV=1E-8) satisfied.

AEM-12-PSEUDO-

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 124.81 |
| AIC (smaller is better) | 130.81 |
| AICC (smaller is better) | 131.86 |
| BIC (smaller is better) | 134.70 |
| CAIC (smaller is better) | 137.70 |
| HQIC (smaller is better) | 131.97 |
| Pearson Chi-Square | 67.20 |
| Pearson Chi-Square / DF | 2.80 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 4.7686 | 0.4201 | 24 | 11.35 | <.0001 |
| conca | B | 5.2611 | 0.5305 | 24 | 9.92 | <.0001 |
| conca | C | -3.4770 | 0.2231 | 24 | -15.59 | <.0001 |
| Resid | | 2.8001 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 156.70 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | >999.999 | 24 | >999.999 | >999.999 |
| B | C | >999.999 | 24 | >999.999 | >999.999 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4.7686 | 0.4201 | 24 | 11.35 | <.0001 | 0.05 | 3.9015 | 5.6356 | 0.9916 | 0.003508 | 0.9802 | 0.9964 |
| B | 5.261' | 0.5305 | 24 | 9.92 | <.0001 | 0.05 | 4.1662 | 6.3561 | 0.9948 | 0.002725 | 0.9847 | 0.9983 |
| C | -3.4770 | 0.2231 | 24 | -15.59 | <.0001 | 0.05 | -3.9374 | -3.0165 | 0.02997 | 0.006487 | 0.01913 | 0.04668 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.4926 | 0.6767 | 24 | -0.73 | 0.4737 | 0.05 | -1.8893 | 0.9041 | 0.611 | 0.151 | 2.470 |
| A | C | 8.2456 | 0.4757 | 24 | 17.33 | <.0001 | 0.05 | 7.2638 | 9.2273 | >999.999 | >999.999 | >999.999 |
| B | C | 8.7381 | 0.5755 | 24 | 15.18 | <.0001 | 0.05 | 7.5503 | 9.9260 | >999.999 | >999.999 | >999.999 |

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This analysis looks at the effect of exposure concentration on zebra musselsurvival for 12 h WT at Lake Carlos

## Zebra mussel mortality - 12 h at Lake Carlos only

ZEH-12-PSEUDO-?

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.CARLOS_12 |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 4202 |
| Number of Trials | 6345 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 51.529434631 | . | 2.309641 |
| 1 | 0 | 3 | 50.064319619 | 1.46511501 | 0.31522 |
| 2 | 0 | 3 | 50.037570263 | 0.02674936 | 0.00862 |
| 3 | 0 | 3 | 50.037552305 | 0.00001796 | 6.941E-6 |

Convergence criterion (ABSGCONV=0.00001) satisfied.

**Fit Statistics**

| | |
|---|---|
| -2 Log Likelihood | 100.06 |
| AIC (smaller is better) | 106.06 |
| AICC (smaller is better) | 107.12 |
| BIC (smaller is better) | 109.96 |
| CAIC (smaller is better) | 112.96 |
| HQIC (smaller is better) | 107.23 |
| Pearson Chi-Square | 32.34 |
| Pearson Chi-Square / DF | 1.35 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 5.0562 | 0.3230 | 24 | 15.66 | <.0001 |
| conca | B | 5.0852 | 0.3229 | 24 | 15.75 | <.0001 |
| conca | C | -3.5634 | 0.1520 | 24 | -23.45 | <.0001 |
| Resid | | 1.3473 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 347.67 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | >999.999 | 24 | >999.999 | >999.999 |
| B | C | >999.999 | 24 | >999.999 | >999.999 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5.0562 | 0.3230 | 24 | 15.66 | <.0001 | 0.05 | 4.3897 | 5.7228 | 0.9937 | 0.002031 | 0.9877 | 0.9967 |
| B | 5.0852 | 0.3229 | 24 | 15.75 | <.0001 | 0.05 | 4.4187 | 5.7517 | 0.9939 | 0.001974 | 0.9881 | 0.9968 |
| C | -3.5634 | 0.1520 | 24 | -23.45 | <.0001 | 0.05 | -3.8770 | -3.2498 | 0.02756 | 0.004073 | 0.02029 | 0.03733 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.02897 | 0.4567 | 24 | -0.06 | 0.9499 | 0.05 | -0.9716 | 0.9136 | 0.971 | 0.378 | 2.493 |
| A | C | 8.6197 | 0.3569 | 24 | 24.15 | <.0001 | 0.05 | 7.8830 | 9.3563 | >999.999 | >999.999 | >999.999 |
| B | C | 8.6486 | 0.3569 | 24 | 24.23 | <.0001 | 0.05 | 7.9120 | 9.3852 | >999.999 | >999.999 | >999.999 |

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This analysis looks at the effect of exposure concentration on zebra musselsurvival for 6 h WT at Shawano Lake

## Zebra mussel mortality - 6 h at Shawano Lake only

AEH-12-PSEUDO-0

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.SHAWANO_6 |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 1676 |
| Number of Trials | 2683 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 133.22145011 | . | 13.08638 |
| 1 | 0 | 3 | 125.83770172 | 7.38374839 | 1.027229 |
| 2 | 0 | 3 | 125.7752597 | 0.06244202 | 0.011145 |
| 3 | 0 | 3 | 125.77525235 | 0.00000735 | 1.8495-6 |

Convergence criterion (ABSGCONV=0.00001) satisfied.

AGH-12-PSEUDO-0

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 251.55 |
| AIC (smaller is better) | 257.55 |
| AICC (smaller is better) | 258.59 |
| BIC (smaller is better) | 261.44 |
| CAIC (smaller is better) | 264.44 |
| HQIC (smaller is better) | 258.71 |
| Pearson Chi-Square | 168.29 |
| Pearson Chi-Square / DF | 7.01 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 1.8319 | 0.2541 | 24 | 7.21 | <.0001 |
| conca | B | 2.4313 | 0.3189 | 24 | 7.62 | <.0001 |
| conca | C | -3.1086 | 0.4511 | 24 | -6.89 | <.0001 |
| Resid | | 7.0121 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 52.53 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | 139.842 | 24 | 48.037 | 407.096 |
| B | C | 254.636 | 24 | 81.422 | 796.341 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1.8319 | 0.2541 | 24 | 7.21 | <.0001 | 0.05 | 1.3075 | 2.3564 | 0.8620 | 0.03023 | 0.7871 | 0.9134 |
| B | 2.4313 | 0.3189 | 24 | 7.62 | <.0001 | 0.05 | 1.7730 | 3.0895 | 0.9192 | 0.02369 | 0.8548 | 0.9565 |
| C | -3.1086 | 0.4511 | 24 | -6.89 | <.0001 | 0.05 | -4.0396 | -2.1776 | 0.04276 | 0.01846 | 0.01730 | 0.1018 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.5993 | 0.4078 | 24 | -1.47 | 0.1546 | 0.05 | -1.4409 | 0.2423 | 0.549 | 0.237 | 1.274 |
| A | C | 4.9405 | 0.5177 | 24 | 9.54 | <.0001 | 0.05 | 3.8720 | 6.0090 | 139.842 | 48.037 | 407.096 |
| B | C | 5.5398 | 0.5524 | 24 | 10.03 | <.0001 | 0.05 | 4.3996 | 6.6800 | 254.636 | 81.422 | 796.341 |

Performed by K. Weber SAS version 9.3 13:52 01MAY14

This analysis looks at the effect of exposure concentration on zebra musselsurvival for 9 h WT at Shawano Lake

AEH-12-PSEUDO-?

## Zebra mussel mortality - 9 h at Shawano Lake only

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.SHAWANO_9 |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 1544 |
| Number of Trials | 2548 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 116.55095602 | . | 13.45866 |
| 1 | 0 | 3 | 106.41799088 | 10.13296514 | 1.431037 |
| 2 | 0 | 3 | 106.21296528 | 0.20502559 | 0.045502 |
| 3 | 0 | 3 | 106.21278193 | 0.00018336 | 0.00006 |
| 4 | 0 | 3 | 106.21278193 | 0.00000000 | 1.03E-10 |

[illegible]-12-PSEUDO-[illegible]

Convergence criterion (GCONV=1E-8) satisfied.

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 212.43 |
| AIC (smaller is better) | 218.43 |
| AICC (smaller is better) | 219.47 |
| BIC (smaller is better) | 222.31 |
| CAIC (smaller is better) | 225.31 |
| HQIC (smaller is better) | 219.58 |
| Pearson Chi-Square | 122.32 |
| Pearson Chi-Square / DF | 5.10 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 2.1523 | 0.2500 | 24 | 8.61 | <.0001 |
| conca | B | 2.8876 | 0.3668 | 24 | 7.87 | <.0001 |
| conca | C | -3.0107 | 0.3526 | 24 | -8.54 | <.0001 |
| Resid | | 5.0965 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 69.66 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | 174.689 | 24 | 71.579 | 426.329 |
| B | C | 364.427 | 24 | 127.514 | >999.999 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2.1523 | 0.2500 | 24 | 8.61 | <.0001 | 0.05 | 1.6362 | 2.6683 | 0.8959 | 0.02332 | 0.8370 | 0.9351 |
| B | 2.8876 | 0.3668 | 24 | 7.87 | <.0001 | 0.05 | 2.1306 | 3.6445 | 0.9472 | 0.01833 | 0.8938 | 0.9745 |
| C | -3.0107 | 0.3526 | 24 | -8.54 | <.0001 | 0.05 | -3.7386 | -2.2829 | 0.04694 | 0.01578 | 0.02324 | 0.09255 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.7353 | 0.4439 | 24 | -1.66 | 0.1106 | 0.05 | -1.6514 | 0.1808 | 0.479 | 0.192 | 1.198 |
| A | C | 5.1630 | 0.4323 | 24 | 11.94 | <.0001 | 0.05 | 4.2708 | 6.0552 | 174.689 | 71.579 | 426.329 |
| B | C | 5.8983 | 0.5088 | 24 | 11.59 | <.0001 | 0.05 | 4.8482 | 6.9484 | 364.427 | 127.514 | >999.999 |

Performed by K. Weber SAS version 9.3 13:52 01MAY14

This analysis looks at the effect of exposure concentration on zebra musselsurvival for 12 h WT at Shawano Lake

## Zebra mussel mortality - 12 h at Shawano Lake only

AEH-12-PSEUDO-...

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.SHAWANO_12 |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 1634 |
| Number of Trials | 2543 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 53.428046326 | . | 2.920853 |
| 1 | 0 | 3 | 51.688258937 | 1.73978739 | 0.343543 |
| 2 | 0 | 3 | 51.661802121 | 0.02645682 | 0.006607 |
| 3 | 0 | 3 | 51.66179312 | 0.00000900 | 2.649E-6 |

Convergence criterion (ABSGCONV=0.00001) satisfied.

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 103.32 |
| AIC (smaller is better) | 109.32 |
| AICC (smaller is better) | 110.37 |
| BIC (smaller is better) | 113.21 |
| CAIC (smaller is better) | 116.21 |
| HQIC (smaller is better) | 110.48 |
| Pearson Chi-Square | 36.18 |
| Pearson Chi-Square / DF | 1.51 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 3.6623 | 0.2781 | 24 | 13.17 | <.0001 |
| conca | B | 3.9379 | 0.3099 | 24 | 12.71 | <.0001 |
| conca | C | -3.2456 | 0.2146 | 24 | -15.12 | <.0001 |
| Resid | | 1.5076 | | | | |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 187.86 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | >999.999 | 24 | 484.394 | >999.999 |
| B | C | >999.999 | 24 | 605.122 | >999.999 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3.6623 | 0.2781 | 24 | 13.17 | <.0001 | 0.05 | 3.0884 | 4.2362 | 0.9750 | 0.006786 | 0.9564 | 0.9857 |
| B | 3.9379 | 0.3099 | 24 | 12.71 | <.0001 | 0.05 | 3.2983 | 4.5776 | 0.9809 | 0.005811 | 0.9644 | 0.9898 |
| C | -3.2456 | 0.2146 | 24 | -15.12 | <.0001 | 0.05 | -3.6886 | -2.8026 | 0.03749 | 0.007744 | 0.02440 | 0.05718 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.2757 | 0.4164 | 24 | -0.66 | 0.5143 | 0.05 | -1.1350 | 0.5837 | 0.759 | 0.321 | 1.793 |
| A | C | 6.9079 | 0.3513 | 24 | 19.67 | <.0001 | 0.05 | 6.1829 | 7.6328 | >999.999 | 484.394 | >999.999 |
| B | C | 7.1835 | 0.3770 | 24 | 19.05 | <.0001 | 0.05 | 6.4054 | 7.9616 | >999.999 | 605.122 | >999.999 |

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This analysis looks at the effect of exposure duration at Lake Carlos
Therefore BI data were excluded from this analysis
Includes 6, 9 and 12 h WWC Lake Carlos data

Zebra mussel mortality - time at Lake Carlos only

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.TIME_CARLOS |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| time | 3 | 6 9 12 |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 81 |
| Number of Observations Used | 81 |
| Number of Events | 12229 |
| Number of Trials | 18649 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 15 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 81 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 9 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 198.68955178 | . | 5.729665 |
| 1 | 0 | 3 | 188.09371633 | 10.59583545 | 1.094281 |
| 2 | 0 | 3 | 187.73020999 | 0.36350634 | 0.063355 |
| 3 | 0 | 3 | 187.72842613 | 0.00178367 | 0.000597 |
| 4 | 0 | 3 | 187.72842604 | 0.00000009 | 3.125E-8 |

[illegible]-12-PSEUDO-[illegible]

Convergence criterion (GCONV=1E-8) satisfied.

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 375.46 |
| AIC (smaller is better) | 393.46 |
| AICC (smaller is better) | 395.99 |
| BIC (smaller is better) | 415.01 |
| CAIC (smaller is better) | 424.01 |
| HQIC (smaller is better) | 402.10 |
| Pearson Chi-Square | 170.24 |
| Pearson Chi-Square / DF | 2.36 |

Parameter Estimates

| Effect | conca | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|
| time | | 6 | -3.7072 | 0.2118 | 72 | -17.50 | <.0001 |
| time | | 9 | -3.4770 | 0.2050 | 72 | -16.96 | <.0001 |
| time | | 12 | -3.5634 | 0.2013 | 72 | -17.70 | <.0001 |
| conca | A | | 8.6197 | 0.4728 | 72 | 18.23 | <.0001 |
| conca | B | | 8.6486 | 0.4728 | 72 | 18.29 | <.0001 |
| conca | C | | 0 | . | . | . | . |
| time*conca | A | 6 | -0.6084 | 0.6036 | 72 | -1.01 | 0.3168 |
| time*conca | B | 6 | -1.1128 | 0.5636 | 72 | -1.97 | 0.0522 |
| time*conca | C | 6 | 0 | . | . | . | . |
| time*conca | A | 9 | -0.3741 | 0.6439 | 72 | -0.58 | 0.5631 |
| time*conca | B | 9 | 0.08949 | 0.7094 | 72 | 0.13 | 0.9000 |
| time*conca | C | 9 | 0 | . | . | . | . |
| time*conca | A | 12 | 0 | . | . | . | . |
| time*conca | B | 12 | 0 | . | . | . | . |
| time*conca | C | 12 | 0 | . | . | . | . |
| Residual | | | 2.3644 | . | . | . | . |

Type III Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| time | 2 | 72 | 5.74 | 0.0048 |
| conca | 2 | 72 | 882.11 | <.0001 |
| time*conca | 4 | 72 | 1.61 | 0.1802 |

Odds Ratio Estimates

| time | conca | time | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|---|---|
| 6 | | 12 | | 0.488 | 72 | 0.292 | 0.815 |
| 9 | | 12 | | 0.992 | 72 | 0.540 | 1.820 |
| | A | | C | >999.999 | 72 | >999.999 | >999.999 |
| | B | | C | >999.999 | 72 | >999.999 | >999.999 |

conca Least Squares Means

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4.7096 | 0.2181 | 72 | 21.60 | <.0001 | 0.05 | 4.2749 | 5.1443 | 0.9911 | 0.001929 | 0.9863 | 0.9942 |

12-PSEUDO

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | 4.7250 | 0.2285 | 72 | 20.68 | <.0001 | 0.05 | 4.2695 | 5.1805 | 0.9912 | 0.001992 | 0.9862 | 0.9944 |
| C | -3.5825 | 0.1190 | 72 | -30.11 | <.0001 | 0.05 | -3.8197 | -3.3454 | 0.02705 | 0.003132 | 0.02146 | 0.03405 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.01537 | 0.3159 | 72 | -0.05 | 0.9613 | 0.05 | -0.6450 | 0.6143 | 0.985 | 0.525 | 1.848 |
| A | C | 8.2922 | 0.2484 | 72 | 33.38 | <.0001 | 0.05 | 7.7970 | 8.7873 | >999.999 | >999.999 | >999.999 |
| B | C | 8.3075 | 0.2576 | 72 | 32.25 | <.0001 | 0.05 | 7.7940 | 8.8211 | >999.999 | >999.999 | >999.999 |

**time Least Squares Means**

| time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 1.4752 | 0.1453 | 72 | 10.15 | <.0001 | 0.05 | 1.1855 | 1.7648 | 0.8138 | 0.02201 | 0.7659 | 0.8538 |
| 9 | 2.1842 | 0.2183 | 72 | 10.01 | <.0001 | 0.05 | 1.7491 | 2.6193 | 0.8988 | 0.01985 | 0.8518 | 0.9321 |
| 12 | 2.1927 | 0.2125 | 72 | 10.32 | <.0001 | 0.05 | 1.7690 | 2.6164 | 0.8996 | 0.01920 | 0.8543 | 0.9319 |

**Differences of time Least Squares Means**

| time | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 9 | -0.7091 | 0.2622 | 72 | -2.70 | 0.0085 | 0.05 | -1.2318 | -0.1864 | 0.492 | 0.292 | 0.830 |
| 6 | 12 | -0.7175 | 0.2575 | 72 | -2.79 | 0.0068 | 0.05 | -1.2308 | -0.2043 | 0.488 | 0.292 | 0.815 |
| 9 | 12 | -0.00845 | 0.3046 | 72 | -0.03 | 0.9780 | 0.05 | -0.6158 | 0.5989 | 0.992 | 0.540 | 1.820 |

**time*conca Least Squares Means**

| conca | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 4.3041 | 0.3096 | 72 | 13.90 | <.0001 | 0.05 | 3.6869 | 4.9213 | 0.9867 | 0.004073 | 0.9756 | 0.9928 |
| B | 6 | 3.8286 | 0.2220 | 72 | 17.24 | <.0001 | 0.05 | 3.3860 | 4.2713 | 0.9787 | 0.004624 | 0.9673 | 0.9862 |
| C | 6 | -3.7072 | 0.2118 | 72 | -17.50 | <.0001 | 0.05 | -4.1294 | -3.2850 | 0.02396 | 0.004953 | 0.01584 | 0.03609 |
| A | 9 | 4.7686 | 0.3860 | 72 | 12.35 | <.0001 | 0.05 | 3.9990 | 5.5381 | 0.9916 | 0.003224 | 0.9820 | 0.9961 |
| B | 9 | 5.2611 | 0.4875 | 72 | 10.79 | <.0001 | 0.05 | 4.2893 | 6.2330 | 0.9948 | 0.002504 | 0.9865 | 0.9980 |
| C | 9 | -3.4770 | 0.2050 | 72 | -16.96 | <.0001 | 0.05 | -3.8857 | -3.0683 | 0.02997 | 0.005961 | 0.02012 | 0.04443 |
| A | 12 | 5.0562 | 0.4278 | 72 | 11.82 | <.0001 | 0.05 | 4.2034 | 5.9091 | 0.9937 | 0.002691 | 0.9853 | 0.9973 |
| B | 12 | 5.0852 | 0.4278 | 72 | 11.89 | <.0001 | 0.05 | 4.2324 | 5.9380 | 0.9939 | 0.002615 | 0.9857 | 0.9974 |
| C | 12 | -3.5634 | 0.2013 | 72 | -17.70 | <.0001 | 0.05 | -3.9647 | -3.1621 | 0.02756 | 0.005395 | 0.01862 | 0.04062 |

**Differences of time*conca Least Squares Means**

| conca | time | _conca | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | B | 6 | 0.4754 | 0.3810 | 72 | 1.25 | 0.2161 | 0.05 | -0.2841 | 1.2349 | 1.609 | 0.753 | 3.438 |
| A | 6 | C | 6 | 8.0113 | 0.3751 | 72 | 21.36 | <.0001 | 0.05 | 7.2635 | 8.7591 | >999.999 | >999.999 | >999.999 |
| A | 6 | A | 9 | -0.4645 | 0.4949 | 72 | -0.94 | 0.3511 | 0.05 | -1.4510 | 0.5220 | 0.628 | 0.234 | 1.685 |
| A | 6 | B | 9 | -0.9571 | 0.5775 | 72 | -1.66 | 0.1018 | 0.05 | -2.1083 | 0.1942 | 0.384 | 0.121 | 1.214 |
| A | 6 | C | 9 | 7.7811 | 0.3713 | 72 | 20.95 | <.0001 | 0.05 | 7.0408 | 8.5213 | >999.999 | >999.999 | >999.999 |
| A | 6 | A | 12 | -0.7522 | 0.5281 | 72 | -1.42 | 0.1587 | 0.05 | -1.8049 | 0.3006 | 0.471 | 0.164 | 1.351 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | B | 12 | -0.7812 | 0.5281 | 72 | -1.48 | 0.1434 | 0.05 | -1.8338 | 0.2715 | 0.458 | 0.160 | 1.312 |
| A | 6 | C | 12 | 7.8675 | 0.3693 | 72 | 21.30 | <.0001 | 0.05 | 7.1313 | 8.6037 | >999.999 | >999.999 | >999.999 |
| B | 6 | C | 6 | 7.5359 | 0.3069 | 72 | 24.56 | <.0001 | 0.05 | 6.9242 | 8.1476 | >999.999 | >999.999 | >999.999 |
| B | 6 | A | 9 | -0.9399 | 0.4453 | 72 | -2.11 | 0.0383 | 0.05 | -1.8277 | -0.05214 | 0.391 | 0.161 | 0.949 |
| B | 6 | B | 9 | -1.4325 | 0.5357 | 72 | -2.67 | 0.0093 | 0.05 | -2.5004 | -0.3646 | 0.239 | 0.082 | 0.694 |
| B | 6 | C | 9 | 7.3056 | 0.3022 | 72 | 24.17 | <.0001 | 0.05 | 6.7032 | 7.9081 | >999.999 | 815.004 | >999.999 |
| B | 6 | A | 12 | -1.2276 | 0.4820 | 72 | -2.55 | 0.0130 | 0.05 | -2.1885 | -0.2667 | 0.293 | 0.112 | 0.766 |
| B | 6 | B | 12 | -1.2566 | 0.4820 | 72 | -2.61 | 0.0111 | 0.05 | -2.2174 | -0.2958 | 0.285 | 0.109 | 0.744 |
| B | 6 | C | 12 | 7.3921 | 0.2997 | 72 | 24.66 | <.0001 | 0.05 | 6.7946 | 7.9895 | >999.999 | 893.003 | >999.999 |
| C | 6 | A | 9 | -8.4758 | 0.4403 | 72 | -19.25 | <.0001 | 0.05 | -9.3536 | -7.5980 | <0.001 | <0.001 | <0.001 |
| C | 6 | B | 9 | -8.9684 | 0.5315 | 72 | -16.87 | <.0001 | 0.05 | -10.0280 | -7.9088 | <0.001 | <0.001 | <0.001 |
| C | 6 | C | 9 | -0.2302 | 0.2948 | 72 | -0.78 | 0.4373 | 0.05 | -0.8178 | 0.3574 | 0.794 | 0.441 | 1.430 |
| C | 6 | A | 12 | -8.7635 | 0.4774 | 72 | -18.36 | <.0001 | 0.05 | -9.7151 | -7.8118 | <0.001 | <0.001 | <0.001 |
| C | 6 | B | 12 | -8.7924 | 0.4774 | 72 | -18.42 | <.0001 | 0.05 | -9.7440 | -7.8409 | <0.001 | <0.001 | <0.001 |
| C | 6 | C | 12 | -0.1438 | 0.2922 | 72 | -0.49 | 0.6241 | 0.05 | -0.7263 | 0.4387 | 0.866 | 0.484 | 1.551 |
| A | 9 | B | 9 | -0.4926 | 0.6219 | 72 | -0.79 | 0.4309 | 0.05 | -1.7322 | 0.7471 | 0.611 | 0.177 | 2.111 |
| A | 9 | C | 9 | 8.2456 | 0.4371 | 72 | 18.86 | <.0001 | 0.05 | 7.3742 | 9.1169 | >999.999 | >999.999 | >999.999 |
| A | 9 | A | 12 | -0.2877 | 0.5763 | 72 | -0.50 | 0.6191 | 0.05 | -1.4364 | 0.8611 | 0.750 | 0.238 | 2.366 |
| A | 9 | B | 12 | -0.3167 | 0.5762 | 72 | -0.55 | 0.5843 | 0.05 | -1.4653 | 0.8320 | 0.729 | 0.231 | 2.298 |
| A | 9 | C | 12 | 8.3320 | 0.4354 | 72 | 19.14 | <.0001 | 0.05 | 7.4641 | 9.1999 | >999.999 | >999.999 | >999.999 |
| B | 9 | C | 9 | 8.7381 | 0.5289 | 72 | 16.52 | <.0001 | 0.05 | 7.6839 | 9.7924 | >999.999 | >999.999 | >999.999 |
| B | 9 | A | 12 | 0.2049 | 0.6486 | 72 | 0.32 | 0.7530 | 0.05 | -1.0881 | 1.4979 | 1.227 | 0.337 | 4.472 |
| B | 9 | B | 12 | 0.1759 | 0.6486 | 72 | 0.27 | 0.7870 | 0.05 | -1.1170 | 1.4689 | 1.192 | 0.327 | 4.344 |
| B | 9 | C | 12 | 8.8246 | 0.6274 | 72 | 16.73 | <.0001 | 0.05 | 7.7731 | 9.8760 | >999.999 | >999.999 | >999.999 |
| C | 9 | A | 12 | -8.5332 | 0.4744 | 72 | -17.99 | <.0001 | 0.05 | -9.4789 | -7.5875 | <0.001 | <0.001 | <0.001 |
| C | 9 | B | 12 | -8.5622 | 0.4744 | 72 | -18.05 | <.0001 | 0.05 | -9.5079 | -7.6166 | <0.001 | <0.001 | <0.001 |
| C | 9 | C | 12 | 0.08642 | 0.2873 | 72 | 0.30 | 0.7644 | 0.05 | -0.4863 | 0.6592 | 1.090 | 0.615 | 1.933 |
| A | 12 | B | 12 | -0.02897 | 0.6050 | 72 | -0.05 | 0.9619 | 0.05 | -1.2350 | 1.1771 | 0.971 | 0.291 | 3.245 |
| A | 12 | C | 12 | 8.6197 | 0.4728 | 72 | 18.23 | <.0001 | 0.05 | 7.8771 | 9.5622 | >999.999 | >999.999 | >999.999 |
| B | 12 | C | 12 | 8.6486 | 0.4728 | 72 | 18.29 | <.0001 | 0.05 | 7.7061 | 9.5911 | >999.999 | >999.999 | >999.999 |

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ACI-12-PSEUDO-0

This analysis looks at the effect of exposure duration at Shawano Lake
Therefore BI data were excluded from this analysis
Includes 6, 9 and 12 h WWC Shawano Lake data
Zebra mussel mortality - time at Shawano Lake only

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.TIME_SHAWANO |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| time | 3 | 6 9 12 |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 81 |
| Number of Observations Used | 81 |
| Number of Events | 4854 |
| Number of Trials | 7774 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 15 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 81 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 9 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 303.20045245 | . | 17.01214 |
| 1 | 0 | 3 | 283.94395153 | 19.25650092 | 1.617625 |
| 2 | 0 | 3 | 283.6500271 | 0.29392443 | 0.039634 |
| 3 | 0 | 3 | 283.6498274 | 0.00019971 | 0.000052 |
| 4 | 0 | 3 | 283.6498274 | 0.00000000 | 8.91E-11 |

AZ 1-12-PSEUDO-4

Convergence criterion (GCONV=1E-8) satisfied.

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 567.30 |
| AIC (smaller is better) | 585.30 |
| AICC (smaller is better) | 587.83 |
| BIC (smaller is better) | 606.85 |
| CAIC (smaller is better) | 615.85 |
| HQIC (smaller is better) | 593.95 |
| Pearson Chi-Square | 326.79 |
| Pearson Chi-Square / DF | 4.54 |

**Parameter Estimates**

| Effect | conca | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|
| time | | 6 | -3.1036 | 0.3629 | 72 | -8.57 | <.0001 |
| time | | 9 | -3.0107 | 0.3328 | 72 | -9.05 | <.0001 |
| time | | 12 | -3.2456 | 0.3724 | 72 | -8.72 | <.0001 |
| conca | A | | 6.9079 | 0.6095 | 72 | 11.33 | <.0001 |
| conca | B | | 7.1835 | 0.6541 | 72 | 10.98 | <.0001 |
| conca | C | | 0 | . | . | . | . |
| time*conca | A | 6 | -1.9673 | 0.7382 | 72 | -2.67 | 0.0095 |
| time*conca | B | 6 | -1.6437 | 0.7908 | 72 | -2.08 | 0.0412 |
| time*conca | C | 6 | 0 | . | . | . | . |
| time*conca | A | 9 | -1.7446 | 0.7334 | 72 | -2.38 | 0.0200 |
| time*conca | B | 9 | -1.2852 | 0.8114 | 72 | -1.58 | 0.1176 |
| time*conca | C | 9 | 0 | . | . | . | . |
| time*conca | A | 12 | 0 | . | . | . | . |
| time*conca | B | 12 | 0 | . | . | . | . |
| time*conca | C | 12 | 0 | . | . | . | . |
| Residual | | | 4.5387 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| time | 2 | 72 | 5.69 | 0.0051 |
| conca | 2 | 72 | 272.74 | <.0001 |
| time*conca | 4 | 72 | 2.20 | 0.0779 |

**Odds Ratio Estimates**

| time | conca | time | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|---|---|
| 6 | | 12 | | 0.344 | 72 | 0.183 | 0.646 |
| 9 | | 12 | | 0.461 | 72 | 0.241 | 0.879 |
| | A | | C | 290.167 | 72 | 165.672 | 508.215 |
| | B | | C | 496.321 | 72 | 268.392 | 917.818 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2.5488 | 0.1916 | 72 | 13.31 | <.0001 | 0.05 | 2.1670 | 2.9307 | 0.9275 | 0.01288 | 0.8972 | 0.9493 |

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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | 3.0856 | 0.2297 | 72 | 13.43 | <.0001 | 0.05 | 2.6277 | 3.5435 | 0.9563 | 0.009600 | 0.9326 | 0.9719 |
| C | -3.1216 | 0.2058 | 72 | -15.17 | <.0001 | 0.05 | -3.5319 | -2.7114 | 0.04222 | 0.008322 | 0.02842 | 0.06230 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.5368 | 0.2991 | 72 | -1.79 | 0.0769 | 0.05 | -1.1330 | 0.05944 | 0.585 | 0.322 | 1.061 |
| A | C | 5.6705 | 0.2811 | 72 | 20.17 | <.0001 | 0.05 | 5.1100 | 6.2309 | 290.167 | 165.672 | 508.215 |
| B | C | 6.2072 | 0.3084 | 72 | 20.13 | <.0001 | 0.05 | 5.5924 | 6.8220 | 496.321 | 268.392 | 917.818 |

**time Least Squares Means**

| time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 0.3849 | 0.1831 | 72 | 2.36 | 0.0210 | 0.05 | 0.05981 | 0.7100 | 0.6951 | 0.03929 | 0.5149 | 0.6704 |
| 9 | 0.6764 | 0.1783 | 72 | 3.79 | 0.0003 | 0.05 | 0.3209 | 1.0319 | 0.6629 | 0.03985 | 0.5795 | 0.7373 |
| 12 | 1.4515 | 0.2709 | 72 | 5.36 | <.0001 | 0.05 | 0.9114 | 1.9916 | 0.8102 | 0.04166 | 0.7133 | 0.8799 |

**Differences of time Least Squares Means**

| time | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 9 | -0.2915 | 0.2416 | 72 | -1.21 | 0.2317 | 0.05 | -0.7732 | 0.1902 | 0.747 | 0.462 | 1.210 |
| 6 | 12 | -1.0667 | 0.3162 | 72 | -3.37 | 0.0012 | 0.05 | -1.6970 | -0.4363 | 0.344 | 0.183 | 0.646 |
| 9 | 12 | -0.7752 | 0.3244 | 72 | -2.39 | 0.0195 | 0.05 | -1.4218 | -0.1286 | 0.461 | 0.24 | 0.879 |

**time*conca Least Squares Means**

| conca | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 1.8319 | 0.2044 | 72 | 8.96 | <.0001 | 0.05 | 1.4244 | 2.2395 | 0.8620 | 0.02432 | 0.8060 | 0.9037 |
| B | 6 | 2.4313 | 0.2566 | 72 | 9.48 | <.0001 | 0.05 | 1.9198 | 2.9428 | 0.9192 | 0.01906 | 0.8721 | 0.9499 |
| C | 6 | -3.1086 | 0.3629 | 72 | -8.57 | <.0001 | 0.05 | -3.8320 | -2.3851 | 0.04276 | 0.01485 | 0.02121 | 0.08432 |
| A | 9 | 2.1523 | 0.2360 | 72 | 9.12 | <.0001 | 0.05 | 1.6819 | 2.6226 | 0.8959 | 0.02201 | 0.8432 | 0.9323 |
| B | 9 | 2.8876 | 0.3461 | 72 | 8.34 | <.0001 | 0.05 | 2.1976 | 3.5776 | 0.9472 | 0.01730 | 0.9000 | 0.9728 |
| C | 9 | -3.0107 | 0.3328 | 72 | -9.05 | <.0001 | 0.05 | -3.6741 | -2.3473 | 0.04694 | 0.01469 | 0.02474 | 0.06728 |
| A | 12 | 3.6623 | 0.4825 | 72 | 7.59 | <.0001 | 0.05 | 2.7005 | 4.6240 | 0.9750 | 0.01177 | 0.9371 | 0.9903 |
| B | 12 | 3.9379 | 0.5378 | 72 | 7.32 | <.0001 | 0.05 | 2.8659 | 5.0100 | 0.9809 | 0.01008 | 0.9461 | 0.9934 |
| C | 12 | -3.2456 | 0.3724 | 72 | -8.72 | <.0001 | 0.05 | -3.9880 | -2.5032 | 0.03749 | 0.01344 | 0.01820 | 0.07564 |

**Differences of time*conca Least Squares Means**

| conca | time | _conca | time | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | B | 6 | -0.5993 | 0.3281 | 72 | -1.83 | 0.0719 | 0.05 | -1.2533 | 0.05466 | 0.549 | 0.286 | 1.056 |
| A | 6 | C | 6 | 4.9405 | 0.4165 | 72 | 11.86 | <.0001 | 0.05 | 4.1102 | 5.7708 | 139.842 | 60.958 | 320.808 |
| A | 6 | A | 9 | -0.3203 | 0.3122 | 72 | -1.03 | 0.3083 | 0.05 | -0.9427 | 0.3020 | 0.726 | 0.390 | 1.353 |
| A | 6 | B | 9 | -1.0556 | 0.4020 | 72 | -2.63 | 0.0105 | 0.05 | -1.8570 | -0.2543 | 0.348 | 0.156 | 0.775 |
| A | 6 | C | 9 | 4.8427 | 0.3906 | 72 | 12.40 | <.0001 | 0.05 | 4.0641 | 5.6213 | 126.809 | 58.213 | 276.236 |
| A | 6 | A | 12 | -1.8303 | 0.5240 | 72 | -3.49 | 0.0008 | 0.05 | -2.8749 | -0.7858 | 0.160 | 0.056 | 0.456 |

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| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | B | 12 | -2.1060 | 0.5753 | 72 | -3.66 | 0.0005 | 0.05 | -3.2529 | -0.9591 | 0.122 | 0.039 | 0.383 |
| A | 6 | C | 12 | 5.0775 | 0.4248 | 72 | 11.95 | <.0001 | 0.05 | 4.2306 | 5.9244 | 160.376 | 68.761 | 374.054 |
| B | 6 | C | 6 | 5.5398 | 0.4445 | 72 | 12.46 | <.0001 | 0.05 | 4.6538 | 6.4258 | 254.636 | 104 986 | 617.605 |
| B | 6 | A | 9 | 0.2790 | 0.3486 | 72 | 0.80 | 0.4261 | 0.05 | -0.4159 | 0.9739 | 1.322 | 0.660 | 2.648 |
| B | 6 | B | 9 | -0.4563 | 0.4308 | 72 | -1.06 | 0.2931 | 0.05 | -1.3152 | 0.4026 | 0.634 | 0 268 | 1.496 |
| D | 6 | C | 9 | 5.4420 | 0.4202 | 72 | 12.95 | <.0001 | 0.05 | 4.6043 | 6.2797 | 230.905 | 99.914 | 533.632 |
| B | 6 | A | 12 | -1.2310 | 0.5464 | 72 | -2.25 | 0.0273 | 0.05 | -2.3203 | -0.1417 | 0.292 | 0.098 | 0.868 |
| B | 6 | B | 12 | -1.5067 | 0.5958 | 72 | -2.53 | 0.0136 | 0.05 | -2.6945 | -0.3189 | 0.222 | 0.068 | 0.727 |
| B | 6 | C | 12 | 5.6768 | 0.4522 | 72 | 12.55 | <.0001 | 0.05 | 4.7753 | 6.5784 | 292.027 | 118.547 | 719.377 |
| C | 6 | A | 9 | -5.2608 | 0.4329 | 72 | -12.15 | <.0001 | 0.05 | -6.1238 | -4.3979 | 0.005 | 0.002 | 0.012 |
| C | 6 | B | 9 | -5.9962 | 0.5015 | 72 | -11.96 | <.0001 | 0.05 | -6.9959 | -4.9964 | 0.002 | <0.001 | 0.007 |
| C | 6 | C | 9 | -0.09783 | 0.4924 | 72 | -0.20 | 0.8431 | 0.05 | -1.0794 | 0.8838 | 0.907 | 0.340 | 2.420 |
| C | 6 | A | 12 | -6.7708 | 0.6037 | 72 | -11.22 | <.0001 | 0.05 | -7.9743 | -5.5674 | 0.001 | <0.001 | 0.004 |
| C | 6 | B | 12 | -7.0465 | 0.6488 | 72 | -10.86 | <.0001 | 0.05 | -8.3398 | -5.7532 | <0.001 | <0.001 | 0.003 |
| C | 6 | C | 12 | 0.1370 | 0.5200 | 72 | 0.26 | 0.7929 | 0.05 | -0.8996 | 1.1736 | 1.147 | 0.407 | 3.234 |
| A | 9 | B | 9 | -0.7363 | 0.4189 | 72 | -1.76 | 0.0834 | 0.05 | -1.5703 | 0.09971 | 0.479 | 0.208 | 1.105 |
| A | 9 | C | 9 | 5.1630 | 0.4079 | 72 | 12.66 | <.0001 | 0.05 | 4.3498 | 5.9762 | 174.689 | 77.461 | 393.957 |
| A | 9 | A | 12 | -1.5100 | 0.5371 | 72 | -2.81 | 0.0063 | 0.05 | -2.5806 | -0.4394 | 0.221 | 0.076 | 0.644 |
| A | 9 | B | 12 | -1.7857 | 0.5873 | 72 | -3.04 | 0.0033 | 0.05 | -2.9563 | -0.6150 | 0.168 | 0.052 | 0.541 |
| A | 9 | C | 12 | 5.3978 | 0.4409 | 72 | 12.24 | <.0001 | 0.05 | 4.5190 | 6.2767 | 220.931 | 91.744 | 532.030 |
| B | 9 | C | 9 | 5.8983 | 0.4801 | 72 | 12.28 | <.0001 | 0.05 | 4.9412 | 6.8555 | 364.427 | 139.934 | 949.066 |
| B | 9 | A | 12 | -0.7747 | 0.5938 | 72 | -1.30 | 0.1961 | 0.05 | -1.9583 | 0.4090 | 0.461 | 0.141 | 1.505 |
| B | 9 | B | 12 | -1.0503 | 0.6395 | 72 | -1.64 | 0.1049 | 0.05 | -2.3252 | 0.2245 | 0.350 | 0.098 | 1.252 |
| B | 9 | C | 12 | 6.1332 | 0.5084 | 72 | 12.06 | <.0001 | 0.05 | 5.1197 | 7.1467 | 460.893 | 167.280 | >999.999 |
| C | 9 | A | 12 | -6.6730 | 0.5861 | 72 | -11.39 | <.0001 | 0.05 | -7.8414 | -5.5046 | 0.001 | <0.001 | 0.004 |
| C | 9 | B | 12 | -6.9487 | 0.6324 | 72 | -10.99 | <.0001 | 0.05 | -8.2094 | -5.6880 | <0.001 | <0.001 | 0.003 |
| C | 9 | C | 12 | 0.2348 | 0.4994 | 72 | 0.47 | 0.6396 | 0.05 | -0.7608 | 1.2305 | 1.265 | 0.467 | 3.423 |
| A | 12 | B | 12 | -0.2757 | 0.7225 | 72 | -0.38 | 0.7039 | 0.05 | -1.7159 | 1.1646 | 0.759 | 0.180 | 3.205 |
| A | 12 | C | 12 | 6.9079 | 0.6095 | 72 | 11.33 | <.0001 | 0.05 | 5.6929 | 8.1228 | >999.999 | 296.753 | >999.999 |
| B | 12 | C | 12 | 7.1835 | 0.6541 | 72 | 10.98 | <.0001 | 0.05 | 5.8795 | 8.4875 | >999.999 | 357.638 | >999.999 |

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**Zebra mussel mortality - BI application**
This analysis only looks at 12h BI at Lake Carlos

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.BI_CARLOS |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 27 |
| Number of Observations Used | 27 |
| Number of Events | 3911 |
| Number of Trials | 6701 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 27 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 202.97656102 | . | 27.20293 |
| 1 | 0 | 3 | 196.1362029 | 6.84035812 | 1.281055 |
| 2 | 0 | 3 | 196.12164269 | 0.01456021 | 0.003436 |
| 3 | 0 | 3 | 196.12164259 | 0.00000010 | 2.497E-8 |

Convergence criterion (GCONV=1E-8) satisfied.

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 392.24 |

| | |
|---|---|
| AIC (smaller is better) | 398.24 |
| AICC (smaller is better) | 399.29 |
| BIC (smaller is better) | 402.13 |
| CAIC (smaller is better) | 405.13 |
| HQIC (smaller is better) | 399.40 |
| Pearson Chi-Square | 245.35 |
| Pearson Chi-Square / DF | 10.22 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 1.4766 | 0.1676 | 24 | 8.81 | <.0001 |
| conca | B | 1.4554 | 0.1685 | 24 | 8.64 | <.0001 |
| conca | C | -3.6173 | 0.4537 | 24 | -7.97 | <.0001 |
| Resid | | 10.2231 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 24 | 71.92 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | 163.019 | 24 | 60.077 | 442.349 |
| B | C | 159.592 | 24 | 58.776 | 433.329 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1.4766 | 0.1676 | 24 | 8.81 | <.0001 | 0.05 | 1.1307 | 1.8225 | 0.8141 | 0.02537 | 0.7560 | 0.8609 |
| B | 1.4554 | 0.1685 | 24 | 8.64 | <.0001 | 0.05 | 1.1076 | 1.8032 | 0.8108 | 0.02585 | 0.7517 | 0.8585 |
| C | -3.6173 | 0.4537 | 24 | -7.97 | <.0001 | 0.05 | -4.5536 | -2.6809 | 0.02615 | 0.01156 | 0.01042 | 0.06411 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | 0.02125 | 0.2377 | 24 | 0.09 | 0.9295 | 0.05 | -0.4693 | 0.5118 | 1.021 | 0.625 | 1.668 |
| A | C | 5.0939 | 0.4837 | 24 | 10.53 | <.0001 | 0.05 | 4.0956 | 6.0921 | 163.019 | 60.077 | 442.349 |
| B | C | 5.0726 | 0.4840 | 24 | 10.48 | <.0001 | 0.05 | 4.0737 | 6.0715 | 159.592 | 58.776 | 433.329 |

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AE-12-PSEUDO-31

**Zebra mussel mortality - BI application**
**This analysis only looks at 12h BI at Shawano Lake**

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.BI_SHAWANO |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |

| | |
|---|---|
| Number of Observations Read | 36 |
| Number of Observations Used | 36 |
| Number of Events | 2782 |
| Number of Trials | 4214 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 3 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 36 |

| Optimization Information | |
|---|---|
| Optimization Technique | Newton-Raphson |
| Parameters in Optimization | 3 |
| Lower Boundaries | 0 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

Iteration History

| Iteration | Restarts | Evaluations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|
| 0 | 0 | 4 | 70.355340511 | . | 2.847558 |
| 1 | 0 | 3 | 69.10008839 | 1.25525212 | 0.156711 |
| 2 | 0 | 3 | 69.090174146 | 0.00991424 | 0.002733 |
| 3 | 0 | 3 | 69.09017162 | 0.00000253 | 8.719E-7 |

Convergence criterion (ABSGCONV=0.00001) satisfied.

| Fit Statistics | |
|---|---|
| -2 Log Likelihood | 138.18 |

ACV-12-PSEUDO-?

| | |
|---|---|
| AIC (smaller is better) | 144.16 |
| AICC (smaller is better) | 144.93 |
| BIC (smaller is better) | 149.93 |
| CAIC (smaller is better) | 151.93 |
| HQIC (smaller is better) | 145.84 |
| Pearson Chi-Square | 38.98 |
| Pearson Chi-Square / DF | 1.18 |

**Parameter Estimates**

| Effect | conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| conca | A | 3.4708 | 0.1683 | 33 | 20.62 | <.0001 |
| conca | B | 4.7103 | 0.3151 | 33 | 14.95 | <.0001 |
| conca | C | -3.0380 | 0.1369 | 33 | -22.18 | <.0001 |
| Resid | | 1.1811 | . | . | . | . |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 3 | 33 | 380.30 | <.0001 |

**Odds Ratio Estimates**

| conca | _conca | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|
| A | C | 671.033 | 33 | 431.554 | >999.999 |
| B | C | >999.999 | 33 | >999.999 | >999.999 |

**conca Least Squares Means**

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3.4708 | 0.1683 | 33 | 20.62 | <.0001 | 0.05 | 3.1284 | 3.8132 | 0.9698 | 0.004922 | 0.9580 | 0.9784 |
| B | 4.7103 | 0.3151 | 33 | 14.95 | <.0001 | 0.05 | 4.0691 | 5.3514 | 0.9911 | 0.002787 | 0.9832 | 0.9953 |
| C | -3.0380 | 0.1369 | 33 | -22.18 | <.0001 | 0.05 | -3.3166 | -2.7594 | 0.04574 | 0.005977 | 0.03501 | 0.05956 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -1.2395 | 0.3573 | 33 | -3.47 | 0.0015 | 0.05 | -1.9663 | -0.5126 | 0.290 | 0.140 | 0.599 |
| A | C | 6.5088 | 0.2170 | 33 | 30.00 | <.0001 | 0.05 | 6.0674 | 6.9502 | 671.033 | 431.554 | >999.999 |
| B | C | 7.7483 | 0.3436 | 33 | 22.55 | <.0001 | 0.05 | 7.0492 | 8.4474 | >999.999 | >999.999 | >999.999 |

Performed by K. Weber SAS version 9.3 13:52 01MAY14

**Zebra mussel mortality - application method**
**This analysis only looks at 12h WT vs 12h BI at Lake Carlos**
**BI only had 12h exposure so can only compare to 12h WT treatment**

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.BI_VS_WT_CARLOS |
| Response Variable (Events) | deac |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Not blocked |
| Estimation Technique | Residual PL |
| Degrees of Freedom Method | Containment |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| conca | 3 | A B C |
| loc | 1 | LC |
| trt_typ | 2 | BI WT |
| tnk | 9 | 1 2 3 4 5 6 7 8 9 |
| tray | 9 | A1 A2 A3 B1 B2 B3 C1 C2 C3 |

| | |
|---|---|
| Number of Observations Read | 54 |
| Number of Observations Used | 54 |
| Number of Events | 8113 |
| Number of Trials | 13046 |

| Dimensions | |
|---|---|
| G-side Cov. Parameters | 2 |
| R-side Cov. Parameters | 1 |
| Columns in X | 11 |
| Columns in Z | 59 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 54 |

| Optimization Information | |
|---|---|
| Optimization Technique | Dual Quasi-Newton |
| Parameters in Optimization | 2 |
| Lower Boundaries | 2 |
| Upper Boundaries | 0 |
| Fixed Effects | Profiled |
| Residual Variance | Profiled |
| Starting From | Data |

| Iteration History | | | | | |
|---|---|---|---|---|---|
| Iteration | Restarts | Subiterations | Objective Function | Change | Max Gradient |

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| | | | | | |
|---|---|---|---|---|---|
| 0 | 0 | 7 | 114.07872109 | 0.76667939 | 5.047E-6 |
| 1 | 0 | 6 | 122.68085509 | 0.38296105 | 0.001385 |
| 2 | 0 | 4 | 123.04352559 | 0.02338733 | 0.000026 |
| 3 | 0 | 3 | 123.07685399 | 0.00172263 | 4.2'2E-6 |
| 4 | 0 | 2 | 123.07422183 | 0.00009370 | 1.151E-7 |
| 5 | 0 | 2 | 123.0743968 | 0.00000662 | 3.075E-7 |
| 6 | 0 | 0 | 123.0743846 | 0.00000000 | 9.59E-6 |

Convergence criterion (PCONV=1.11022E-8) satisfied.

| Fit Statistics | |
|---|---|
| -2 Res Log Pseudo-Likelihood | 123.07 |
| Generalized Chi-Square | 60.23 |
| Gener. Chi-Square / DF | 1.25 |

**Covariance Parameter Estimates**

| Cov Parm | Estimate | Standard Error |
|---|---|---|
| tnk | 0.05595 | 0.07644 |
| tray(tnk) | 0.2462 | 0.1059 |
| Residual (VC) | 1.2548 | 0.4968 |

**Solutions for Fixed Effects**

| Effect | conca | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|
| conca | A | | 4.97'1 | 0.3726 | 2 | 13.34 | 0.0056 |
| conca | B | | 4.9871 | 0.3757 | 2 | 13.27 | 0.0056 |
| conca | C | | -3.5461 | 0.2591 | 2 | -13.68 | 0.0053 |
| trt_typ | | BI | -0.09125 | 0.3519 | 2 | -0.26 | 0.8197 |
| trt_typ | | WT | 0 | . | . | . | . |
| conca*trt_typ | A | BI | -3.2699 | 0.5028 | 2 | -6.50 | 0.0228 |
| conca*trt_typ | A | WT | 0 | . | . | . | . |
| conca*trt_typ | B | BI | -3.2397 | 0.5887 | 2 | -5.50 | 0.0315 |
| conca*trt_typ | B | WT | 0 | . | . | . | . |
| conca*trt_typ | C | BI | 0 | . | . | . | . |
| conca*trt_typ | C | WT | 0 | . | . | . | . |

**Odds Ratio Estimates**

| conca | trt_typ | _conca | _trt_typ | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|---|---|
| A | | C | | 974.744 | 2 | 251.874 | >999.999 |
| B | | C | | >999.999 | 2 | 316.839 | >999.999 |
| | BI | | WT | 0.104 | 2 | 0.043 | 0.254 |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 2 | 2 | 427.24 | 0.0023 |
| trt_typ | 1 | 2 | 119.73 | 0.0082 |
| conca*trt_typ | 2 | 2 | 24.60 | 0.0391 |

**conca Least Squares Means**

| | | | | | | Standard Error | Lower | Upper |
|---|---|---|---|---|---|---|---|---|

| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Mean | Mean | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3.2905 | 0.2483 | 2 | 13.25 | 0.0056 | 0.05 | 2.2222 | 4.3588 | 0.9641 | 0.008593 | 0.9022 | 0.9874 |
| B | 3.3216 | 0.2256 | 2 | 14.72 | 0.0046 | 0.05 | 2.3508 | 4.2925 | 0.9652 | 0.007586 | 0.9130 | 0.9865 |
| C | -3.5917 | 0.1931 | 2 | -18.60 | 0.0029 | 0.05 | -4.4224 | -2.7610 | 0.02681 | 0.005038 | 0.01186 | 0.05947 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.03115 | 0.3355 | 2 | -0.09 | 0.9345 | 0.05 | -1.4747 | 1.4124 | 0.969 | 0.229 | 4.[illegible]06 |
| A | C | 6.8822 | 0.3145 | 2 | 21.88 | 0.0021 | 0.05 | 5.5289 | 8.2354 | 974.744 | 251.874 | >999.999 |
| B | C | 6.9133 | 0.2684 | 2 | 25.76 | 0.0015 | 0.05 | 5.7584 | 8.0683 | >999.999 | 316.839 | >999.999 |

**trt_typ Least Squares Means**

| trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BI | -0.1237 | 0.1379 | 2 | -0.90 | 0.4641 | 0.05 | -0.7[illegible]69 | 0.4694 | 0.4691 | 0.03433 | 0.3261 | 0.6152 |
| WT | 2.1374 | 0.1981 | 2 | 10.79 | 0.0085 | 0.05 | 1.2852 | 2.9895 | 0.8945 | 0.01869 | 0.7833 | 0.9521 |

**Differences of trt_typ Least Squares Means**

| trt_typ | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BI | WT | -2.2611 | 0.2066 | 2 | -10.94 | 0.0082 | 0.05 | -3.1502 | -1.3720 | 0.104 | 0.043 | 0.254 |

**conca*trt_typ Least Squares Means**

| conca | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BI | 1.6099 | 0.2211 | 2 | 7.28 | 0.0183 | 0.05 | 0.6585 | 2.5613 | 0.8334 | 0.03070 | 0.6589 | 0.9283 |
| A | WT | 4.9711 | 0.3726 | 2 | 13.34 | 0.0056 | 0.05 | 3.3677 | 6.5744 | 0.9931 | 0.002549 | 0.9667 | 0.9986 |
| B | BI | 1.6562 | 0.2218 | 2 | 7.47 | 0.0175 | 0.05 | 0.7017 | 2.6106 | 0.8397 | 0.02986 | 0.6686 | 0.9315 |
| B | WT | 4.9871 | 0.3757 | 2 | 13.27 | 0.0056 | 0.05 | 3.3704 | 6.6038 | 0.9932 | 0.002530 | 0.9668 | 0.9986 |
| C | BI | -3.6373 | 0.2633 | 2 | -13.81 | 0.0052 | 0.05 | -4.7702 | -2.5044 | 0.02565 | 0.006580 | 0.008407 | 0.07555 |
| C | WT | -3.5461 | 0.2591 | 2 | -13.68 | 0.0053 | 0.05 | -4.6610 | -2.4311 | 0.02803 | 0.007060 | 0.009359 | 0.08083 |

**Differences of conca*trt_typ Least Squares Means**

| conca | trt_typ | _conca | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BI | A | WT | -3.3611 | 0.3591 | 2 | -9.36 | 0.0112 | 0.05 | -4.9061 | -1.8162 | 0.035 | 0.007 | 0.163 |
| A | BI | B | BI | -0.04624 | 0.3132 | 2 | -0.15 | 0.8962 | 0.05 | -1.3939 | 1.3014 | 0.955 | 0.248 | 3.674 |
| A | BI | B | WT | -3.3772 | 0.4360 | 2 | -7.75 | 0.0163 | 0.05 | -5.2531 | -1.5013 | 0.034 | 0.005 | 0.223 |
| A | BI | C | BI | 5.2472 | 0.3438 | 2 | 15.26 | 0.0043 | 0.05 | 3.7678 | 6.7267 | 190.040 | 43.285 | 834.356 |
| A | BI | C | WT | 5.1560 | 0.3406 | 2 | 15.14 | 0.0043 | 0.05 | 3.6903 | 6.6217 | 173.466 | 40.057 | 751.192 |
| A | WT | B | BI | 3.3149 | 0.4337 | 2 | 7.64 | 0.0167 | 0.05 | 1.4490 | 5.1808 | 27.520 | 4.259 | 177.829 |
| A | WT | B | WT | -0.01606 | 0.5292 | 2 | -0.03 | 0.9785 | 0.05 | -2.2930 | 2.2609 | 0.984 | 0.101 | 9.591 |
| A | WT | C | BI | 8.6084 | 0.4563 | 2 | 18.87 | 0.0028 | 0.05 | 6.6452 | 10.5716 | >999.999 | 769.053 | >999.999 |
| A | WT | C | WT | 8.5171 | 0.4539 | 2 | 18.77 | 0.0028 | 0.05 | 6.5642 | 10.4700 | >999.999 | 709.275 | >999.999 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | BI | B | WT | -3.3310 | 0.4209 | 2 | -7.91 | 0.0156 | 0.05 | -5.1419 | -1.5200 | 0.036 | 0.006 | 0.219 |
| B | BI | C | BI | 5.2935 | 0.3390 | 2 | 15.61 | 0.0041 | 0.05 | 3.8348 | 6.7521 | 199.033 | 46.285 | 855.878 |
| B | BI | C | WT | 5.2022 | 0.3027 | 2 | 17.19 | 0.0034 | 0.05 | 3.8999 | 6.5045 | 181.674 | 49.397 | 668.169 |
| B | WT | C | BI | 8.6244 | 0.4245 | 2 | 20.32 | 0.0024 | 0.05 | 6.7980 | 10.4508 | >999.999 | 896.092 | >999.999 |
| B | WT | C | WT | 8.5332 | 0.4500 | 2 | 18.96 | 0.0028 | 0.05 | 6.5971 | 10.4693 | >999.999 | 732.963 | >999.999 |
| C | BI | C | WT | -0.09125 | 0.3519 | 2 | -0.26 | 0.8197 | 0.05 | -1.6055 | 1.4230 | 0.913 | 0.201 | 4.149 |

Performed by K. Weber SAS version 9.3 13:52 01MAY14

Zebra mussel mortality - application method
This analysis only looks at 12h WT vs 12h BI at Shawano Lake
BI only had 12h exposure so can only compare to 12h WT treatment

The GLIMMIX Procedure

| Model Information | |
|---|---|
| Data Set | WORK.BI_VS_WT_SHAWANO |
| Response Variable (Events) | dead |
| Response Variable (Trials) | tot |
| Response Distribution | Binomial |
| Link Function | Logit |
| Variance Function | Default |
| Variance Matrix | Not blocked |
| Estimation Technique | Residual PL |
| Degrees of Freedom Method | Containment |

Class Level Information

| Class | Levels | Values |
|---|---|---|
| conca | 3 | A B C |
| loc | 1 | SL |
| trt_typ | 2 | BI WT |
| tnk | 9 | 1 2 3 4 5 6 7 8 9 |
| tray | 9 | B2 A2 A3 B1 B2 B3 C1 C2 C3 |

| | |
|---|---|
| Number of Observations Read | 63 |
| Number of Observations Used | 63 |
| Number of Events | 4416 |
| Number of Trials | 6757 |

| Dimensions | |
|---|---|
| G-side Cov. Parameters | 2 |
| R-side Cov. Parameters | 1 |
| Columns in X | 11 |
| Columns in Z | 57 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 63 |

| Optimization Information | |
|---|---|
| Optimization Technique | Dual Quasi-Newton |
| Parameters in Optimization | 2 |
| Lower Boundaries | 2 |
| Upper Boundaries | 0 |
| Fixed Effects | Profiled |
| Residual Variance | Profiled |
| Starting From | Data |

Iteration History

| Iteration | Restarts | Subiterations | Objective Function | Change | Max Gradient |
|---|---|---|---|---|---|

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| | | | | | |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 117.93087772 | 0.33463315 | 33.76522 |
| 1 | 0 | 0 | 137.37063376 | 0.08112774 | 34.63360 |
| 2 | 0 | 0 | 139.77951899 | 0.00202242 | 36.64421 |
| 3 | 0 | 0 | 139.82024805 | 0.00000083 | 35.66795 |
| 4 | 0 | 0 | 139.82026165 | 0.00000000 | 35.66796 |

Convergence criterion (PCONV=1.11022E-8) satisfied.

**Estimated G matrix is not positive definite.**

**Fit Statistics**

| | |
|---|---|
| -2 Res Log Pseudo-Likelihood | 139.82 |
| Generalized Chi-Square | 75.16 |
| Gener. Chi-Square / DF | 1.32 |

**Covariance Parameter Estimates**

| Cov Parm | Estimate | Standard Error |
|---|---|---|
| tnk | 0 | . |
| tray(tnk) | 0 | . |
| Residual (VC) | 1.3186 | 0.2470 |

**Solutions for Fixed Effects**

| Effect | conca | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|
| conca | A | | 3.6623 | 0.2600 | 10 | 14.08 | <.0001 |
| conca | B | | 3.9379 | 0.2899 | 10 | 13.58 | <.0001 |
| conca | C | | -3.2456 | 0.2007 | 10 | -16.17 | <.0001 |
| trt_typ | | BI | 0.2076 | 0.2474 | 10 | 0.84 | 0.4212 |
| trt_typ | | WT | 0 | . | . | . | . |
| conca*trt_typ | A | BI | -0.3990 | 0.4006 | 10 | -1.00 | 0.3427 |
| conca*trt_typ | A | WT | 0 | . | . | . | . |
| conca*trt_typ | B | BI | 0.5648 | 0.5061 | 10 | 1.12 | 0.2905 |
| conca*trt_typ | B | WT | 0 | . | . | . | . |
| conca*trt_typ | C | BI | 0 | . | . | . | . |
| conca*trt_typ | C | WT | 0 | . | . | . | . |

**Odds Ratio Estimates**

| conca | trt_typ | _conca | trt_typ | Estimate | DF | 95% Confidence Limits | |
|---|---|---|---|---|---|---|---|
| A | | C | | 819.206 | 10 | 524.298 | >999.999 |
| B | | C | | >999.999 | 10 | 994.356 | >999.999 |
| | BI | | WT | 1.301 | 10 | 0.835 | 2.025 |

**Type III Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| conca | 2 | 10 | 767.73 | <.0001 |
| trt_typ | 1 | 10 | 1.75 | 0.2154 |
| conca*trt_typ | 2 | 10 | 1.60 | 0.2494 |

**conca Least Squares Means**

| | | | | | | | | | Standard Error | Lower | Upper |
|---|---|---|---|---|---|---|---|---|---|---|---|

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| conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Mean | Mean | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3.5665 | 0.1575 | 10 | 22.64 | <.0001 | 0.05 | 3.2156 | 3.9175 | 0.9725 | 0.004209 | 0.9614 | 0.9805 |
| B | 4.3241 | 0.2207 | 10 | 19.59 | <.0001 | 0.05 | 3.8323 | 4.8159 | 0.9869 | 0.002848 | 0.9788 | 0.9920 |
| C | -3.1418 | 0.1237 | 10 | -25.39 | <.0001 | 0.05 | -3.4175 | -2.8661 | 0.04142 | 0.004912 | 0.03175 | 0.05385 |

**Differences of conca Least Squares Means**

| conca | _conca | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | -0.7576 | 0.2712 | 10 | -2.79 | 0.0190 | 0.05 | -1.3618 | -0.1534 | 0.469 | 0.256 | 0.858 |
| A | C | 6.7083 | 0.2003 | 10 | 33.49 | <.0001 | 0.05 | 6.2621 | 7.1546 | 819.206 | 524.298 | >999.999 |
| B | C | 7.4659 | 0.2530 | 10 | 29.51 | <.0001 | 0.05 | 6.9021 | 8.0297 | >999.999 | 994.356 | >999.999 |

**trt_typ Least Squares Means**

| trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BI | 1.7144 | 0.1348 | 10 | 12.72 | <.0001 | 0.05 | 1.4141 | 2.0146 | 0.8474 | 0.01743 | 0.8044 | 0.8823 |
| WT | 1.4515 | 0.1460 | 10 | 9.94 | <.0001 | 0.05 | 1.1262 | 1.7769 | 0.8102 | 0.02245 | 0.7551 | 0.8553 |

**Differences of trt_typ Least Squares Means**

| trt_typ | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BI | WT | 0.2628 | 0.1987 | 10 | 1.32 | 0.2154 | 0.05 | -0.1799 | 0.7056 | 1.301 | 0.835 | 2.025 |

**conca*trt_typ Least Squares Means**

| conca | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Mean | Standard Error Mean | Lower Mean | Upper Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BI | 3.4708 | 0.1778 | 10 | 19.52 | <.0001 | 0.05 | 3.0746 | 3.8670 | 0.9698 | 0.005200 | 0.9558 | 0.9795 |
| A | WT | 3.6623 | 0.2600 | 10 | 14.08 | <.0001 | 0.05 | 3.0829 | 4.2417 | 0.9750 | 0.006346 | 0.9562 | 0.9858 |
| B | BI | 4.7103 | 0.3330 | 10 | 14.15 | <.0001 | 0.05 | 3.9684 | 5.4522 | 0.9911 | 0.002944 | 0.9814 | 0.9957 |
| B | WT | 3.9379 | 0.2899 | 10 | 13.59 | <.0001 | 0.05 | 3.2921 | 4.5838 | 0.9809 | 0.005435 | 0.9642 | 0.9899 |
| C | BI | -3.0380 | 0.1447 | 10 | -21.00 | <.0001 | 0.05 | -3.3604 | -2.7156 | 0.04574 | 0.006315 | 0.03356 | 0.06206 |
| C | WT | -3.2456 | 0.2007 | 10 | -16.17 | <.0001 | 0.05 | -3.6928 | -2.7983 | 0.03749 | 0.007242 | 0.02430 | 0.05741 |

**Differences of conca*trt_typ Least Squares Means**

| conca | trt_typ | _conca | trt_typ | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Alpha | Lower | Upper | Odds Ratio | Lower Confidence Limit for Odds Ratio | Upper Confidence Limit for Odds Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BI | A | WT | -0.1915 | 0.3150 | 10 | -0.61 | 0.5569 | 0.05 | -0.8934 | 0.5104 | 0.826 | 0.409 | 1.666 |
| A | BI | B | BI | -1.2395 | 0.3775 | 10 | -3.28 | 0.0082 | 0.05 | -2.0805 | -0.3984 | 0.290 | 0.125 | 0.671 |
| A | BI | B | WT | -0.4671 | 0.3400 | 10 | -1.37 | 0.1995 | 0.05 | -1.2248 | 0.2906 | 0.627 | 0.294 | 1.337 |
| A | BI | C | BI | 6.5088 | 0.2292 | 10 | 28.39 | <.0001 | 0.05 | 5.9980 | 7.0196 | 671.033 | 402.634 | >999.999 |
| A | BI | C | WT | 6.7164 | 0.2682 | 10 | 25.05 | <.0001 | 0.05 | 6.1189 | 7.3139 | 825.827 | 454.360 | >999.999 |
| A | WT | B | BI | -1.0480 | 0.4225 | 10 | -2.48 | 0.0325 | 0.05 | -1.9893 | -0.1067 | 0.351 | 0.137 | 0.899 |
| A | WT | B | WT | -0.2757 | 0.3894 | 10 | -0.71 | 0.4952 | 0.05 | -1.1433 | 0.5920 | 0.759 | 0.319 | 1.808 |
| A | WT | C | BI | 6.7003 | 0.2976 | 10 | 22.52 | <.0001 | 0.05 | 6.0372 | 7.3633 | 812.639 | 418.730 | >999.999 |
| A | WT | C | WT | 6.9079 | 0.3285 | 10 | 21.03 | <.0001 | 0.05 | 6.1759 | 7.6398 | >999.999 | 481.020 | >999.999 |

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| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | BI | B | WT | 0.7723 | 0.4415 | 10 | 1.75 | 0.1108 | 0.05 | -0.2113 | 1.7560 | 2.165 | 0.810 | 5.789 |
| B | BI | C | BI | 7.7483 | 0.3630 | 10 | 21.34 | <.0001 | 0.05 | 6.9394 | 8.5572 | >999.999 | >999.999 | >999.999 |
| B | BI | C | WT | 7.9559 | 0.3888 | 10 | 20.46 | <.0001 | 0.05 | 7.0896 | 8.8221 | >999.999 | >999.999 | >999.999 |
| B | WT | C | BI | 6.9759 | 0.3240 | 10 | 21.53 | <.0001 | 0.05 | 6.2541 | 7.6978 | >999.999 | 520.145 | >999.999 |
| B | WT | C | WT | 7.1836 | 0.3526 | 10 | 20.37 | <.0001 | 0.05 | 6.3979 | 7.9691 | >999.999 | 600.599 | >999.999 |
| C | BI | C | WT | 0.2076 | 0.2474 | 10 | 0.84 | 0.4212 | 0.05 | -0.3438 | 0.7589 | 1.231 | 0.709 | 2.136 |

Performed by K. Weber SAS version 9.3 13:52 01MAY14

KLW
1 MAY 2014

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