

**Animas River
Sediment Toxicity Testing Report
December 2012 Sediment Collection**

Prepared for:



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Acronym List

BLM	Bureau of Land Management
°C	Degrees Celsius
CDPHE	Colorado Department of Public Health and Environment
CETIS	Comprehensive Environmental Toxicity Information System
Control N	Negative laboratory control
Control P	Positive laboratory control
DO	Dissolved Oxygen
EC ₅₀	50% Median Effective Concentration
EPA	Environmental Protection Agency
ESAT	Environmental Services Assistance Team
g	Grams
gpm	Gallons per minute
kg	Kilogram
LC ₅₀	50% Lethal Concentration
MHRW	Moderately Hard Reconstituted Water
mg	Milligram
mL	Milliliter
NH ₃	Ammonia
QAPP	Quality Assurance Project Plan
SAP	Sampling and Analysis Plan
SGC	Sunnyside Gold Corporation
SOP	Standard Operating Procedure
TAC	Test Acceptability Criterion
YCT	Yeast, Cerophyl, and Trout Chow
ZnSO ₄	Zinc sulfate heptahydrate

1.0 INTRODUCTION

A 10-day, static-renewal sediment toxicity test using the amphipod, (*Hyaella azteca*), and sediment from the Animas River (San Juan County, Colorado) was performed at the United States Environmental Protection Agency (EPA) Region 8 Laboratory in December 2012. The purpose of this toxicity test was to determine the toxicity of sediments collected from the Animas River by assessing survival and growth. A 96-hour reference toxicity test was performed concurrently with the Animas River toxicity test as a quality assurance measure. The reference toxicity test consisted of an aqueous stock solution spiked with zinc, with a test endpoint of survival. This report includes a brief background of the Animas Mining District (Section 1.1), materials and methods (Section 2.0), testing results (Section 3.0), a discussion of results (Section 4.0), and supporting references (Section 5.0).

1.1 Background

Information in this section was obtained from the *Final 2012 Sampling and Analysis Plan/Quality Assurance Project Plan, Revision 1, Upper Animas Mining District Gladstone, San Juan County, Colorado*, dated September 2012 (ESAT, 2012).

The discovery of gold and silver brought miners to the Silverton area and Animas Mining District in the early 1870's. The discovery of silver in the base-metal ores was the major factor in establishing Silverton as a permanent settlement. Between 1870 and 1890, the richer ore deposits were discovered and mined to the extent possible. Not until 1890 was any serious attempt made to mine and concentrate the larger low-grade ore bodies in the area. By 1900, there were 12 concentration mills in the valley sending products to the Kendrick and Gelder Smelter near the mouth of Cement Creek. Mining and milling operations slowed down circa 1905, and the mines were consolidated into fewer and larger operations with the facilities for milling large volumes of ore. After 1907, mining and milling continued throughout the basin whenever prices were relatively favorable. Gladstone, located about eight miles upstream of Silverton on Cement Creek, is the site of an historic mining town developed in the 1880s commensurate with the onset of mining in the surrounding area. The town was the central location and railroad terminus for the milling and shipping of mine ores from the surrounding three-square-mile valley. The town declined in the 1920's and no remnants of the town remain. By the 1970's, the Sunnyside Mine was the only year-round producing mine remaining in the county. This mine ceased production in 1991, and has since undergone reclamation efforts. The Gold King Mine's permit with DRMS is currently in inactive status; however, landowners hope to rehabilitate the mine.

Both the Sunnyside and Gold King properties were partially accessed through the American Tunnel that has its portal in Gladstone. Previously the American Tunnel drained as much as 1,600 gallons per minute (gpm) of water from the mines. A lime feed and settling pond-type treatment facility was constructed in Gladstone in 1979 by Standard Metals Corporation. Water discharging from the American Tunnel was treated as required by the water discharge permit. The facility operations and mine ownership

was later transferred to the Sunnyside Gold Corporation (SGC). Under jurisdiction of a court consent decree to terminate their discharge permit, SGC installed several bulkheads within the Sunnyside Mine that greatly reduced the amount of discharge from the American Tunnel. Seventy to one hundred gpm continue to discharge, presumably from near surface groundwater.

In January 2003 the treatment facility, operations, and permit were transferred to the Gold King Mines Corporation. The settling ponds were deeded to the San Juan Corporation by SGC prior to the lease between the Gold King Mines and San Juan Corporations. The treatment facility continued to treat the remaining American Tunnel discharge and the Gold King discharge until September 2004. The San Juan Corporation required SGC to reclaim the four settling ponds (completed in 2005) following termination of the San Juan Corporation and SGC lease. The Gold King Mines Corporation was subsequently evicted and the balance of the Gold King Mines Corporation land was acquired by the San Juan Corporation as the lien holder. The American Tunnel portal reclamation and removal of some out buildings were completed in 2006. The Bureau of Land Management (BLM) manages land associated with the American Tunnel portal and vicinity; however, the San Juan Corporation owns the majority of the land surrounding the portal.

Numerous historic and now abandoned mines exist within a two-mile radius of Gladstone. They include: the Upper Gold King 7 Level, American Tunnel, Grand Mogul, Mogul, and Red and Bonita, Evelyne, Henrietta, Joe and John, and Lark mines. Some of these mines have acid mine drainage that flows between 30 and 300 gpm directly or indirectly into Cement Creek and eventually into the Animas River.

1.2 Objective

The objectives of this toxicity test were to (a) support the yearly monitoring activities at the Animas River, (b) characterize the effects of mine waste-impacted sediment samples on *H. azteca* under subchronic exposure conditions, and (c) generate data to support development of the future Baseline Ecological Risk Assessment and Remedial Investigation.

2.0 MATERIALS AND METHODS

This section outlines the materials and methods used for testing purposes, including sediment collection procedures, water preparation and delivery, test organisms, food preparation, and testing procedures. General test methods following EPA (2000) are discussed below and summarized in **Table 2.0-1**.

2.1 Study Design

The 10-day Animas River sediment toxicity test followed protocols listed in EPA Method 100.1 (EPA, 2000). *H. azteca* survival and growth were measured after the exposure period. The test used a negative laboratory control (Control N; Horsecreek Reservoir control sediment) and a positive laboratory control (Control P; laboratory control sediment spiked with 1,000 milligrams per kilogram [mg/kg] zinc) to help evaluate the

overall health of the test organisms and to provide a baseline growth measurement for amphipods exposed to clean sediment. Eight replicates for each sample location and each laboratory control were used during the 10 day toxicity test.

Site sediment was thoroughly homogenized in a stainless steel pan before it was distributed into test chambers one day before the organisms were introduced. 100 milliliters (mL) of sediment was placed in each test replicate chamber before they were placed into a temperature-controlled water bath.

The water bath temperature was held at $23 \pm 2^{\circ}\text{C}$ for the duration of the test and met the performance criterion. According to EPA Method 100.1 (EPA, 2000) the daily mean test temperature should be $\pm 1^{\circ}\text{C}$ and the instantaneous must always be within $\pm 3^{\circ}\text{C}$ of the target temperature of 23°C . Moderately Hard Reconstituted Water (MHRW) was added to each test chamber before ten organisms were counted, verified, and introduced. One mL of Yeast, Cerophyl®, and Trout Chow (YCT) feed mixture was added to each test chamber daily and the overlying water was renewed at a rate of two volumes (350 mL) per day for the 10-day test period.

The water quality measurements were collected daily as described in **Exhibit 1** (below). The water quality parameters pH, conductivity, and hardness were checked on test Day 0 and test Day 9. Dissolved Oxygen (DO) and temperature were measured daily. A syringe was used to collect ammonia samples in overlying water from each replicate on the first and last day of the test. On Day 10 of the test, temperature and DO were measured in each test chamber before samples for overlying surface water were collected as a composite sample from all replicates. All ammonia samples were inspected for the presence of test organisms before the samples were prepared for analysis to ensure no organisms were inadvertently removed from the test chamber.

Exhibit 1: Activities Schedule for a 10-Day Sediment Toxicity Test

Day	Activity
Day -1	Add sediment into test chambers and start renewal of overlying water.
Day 0	Measure surface water quality parameters (i.e., pH, temperature, DO, conductivity, and ammonia) in each replicate. Obtain hardness measurement by collecting a composite sample from all replicates. Collect pore water samples for dissolved metals analysis from each replicate. Transfer 10 organisms into each test chamber and release them under the surface of the water to avoid entrapment. Add 1.0 mL of YCT into each test chamber. Obtain 80 additional test organisms to measure initial dry weight.
Day 1 through 8	Feed organisms 1.0 mL YCT and measure temperature and DO in each test chamber.
Day 9	Measure surface water quality (i.e., pH, temperature, DO, conductivity, and ammonia) for each replicate. Collect a composite sample from all replicates for hardness measurement. Add 1.0 mL of YCT into each test chamber.
Day 10	Measure temperature and DO. Collect the surviving organisms from each replicate.

2.2 Sediment Collection

Composite sediment samples were collected in December 2012 from the Animas River, Cement Creek, and Mineral Creek in accordance with the 2012 Sampling and Analysis Plan/Quality Assurance Project Plan (SAP/QAPP) (ESAT, 2012). Sediment was collected from a depth of 0-2 inches using a Teflon hand trowel. The sample containers were placed on ice until received at the EPA Region 8 laboratory before they were placed in a 4°C cooler for preservation. Sample collection equipment was decontaminated between each sampling location in accordance with procedures outlined in the SAP/QAPP (ESAT, 2012).

2.3 Test Water Preparation and Delivery

MHRW was prepared in accordance with Smith *et al.* (1997) by adding 47.4 grams (g) of calcium sulfate, 122.8 g of magnesium sulfate, 48 g of sodium bicarbonate, and 4 g of potassium chloride to the laboratory stainless steel batch tank containing 1,000 L of deionized water. The batch tank was continuously aerated for the duration of the toxicity test after the MHRW was prepared. Water quality was measured to verify that the following parameters were met: hardness between 90 and 100 milligrams per liter (mg/L), conductivity between 330 and 360 microsiemens/centimeter, and pH between 7.8 and 8.2 standard units (EPA, 2000). **Table 2.3-1** summarizes the parameters for the MHRW used in the test, and the resulting pH and hardness. The MHRW was delivered to each test chamber at a rate of two volumes (approximately 350 mL) of overlying water per day using a glass distribution box similar to the one described in Benoit *et al.* (1983).

Table 2.3-1 Moderately Hard Reconstituted Water Composition and Chemistry

CaSO ₄	MgSO ₄ ·7H ₂ O	NaHCO ₃	KCl	Final pH*	Final Hardness*
47.4 g/1000 L	122.8 g/1000 L	48 g/1000 L	4 g/1000 L	7.47	73 mg/L

*an average was taken from two batches

2.4 Test Organisms

The juvenile amphipods needed for the sediment toxicity test were obtained from Aquatic Biosystems (Ft. Collins, Colorado). The organisms were kept in their shipping bag after they arrived at the laboratory and placed in a holding tank for about 48 hours for temperature acclimation. Water acclimation was not a concern because the organisms had been cultured and shipped in MHRW. Regardless, the shipping bag was slowly opened to allow a small amount of laboratory MHRW to mix with the shipping water. This procedure was repeated several times through the course of one day until laboratory MHRW and shipping water were well mixed. Test organisms were approximately 7 to 10-days old at the time of testing.

2.5 Test Food Preparation

Organisms were fed a YCT mixture daily (see **Table 2.5-1**). YCT was prepared by adding 5 g of Trout Chow® to 1 L of deionized water, followed by homogenization in a blender (EPA, 2000). The homogenized mixture was poured into a 2-L separatory

funnel, aerated, and allowed to digest for one week at room temperature. The aeration apparatus was removed after the digestion period. The solid material settled out for one hour, after which the supernatant was collected using a 110 mesh Nitex screen. Yeast solution was prepared by adding 5 g of dry yeast to 1 L deionized water, followed by mixing. Cerophyl® was prepared by adding 5 g of alfalfa pellets to 1 L of deionized water, followed by homogenization in a blender. Equal parts of yeast, Trout Chow (supernatant), and Cerophyl® solutions were then added to a beaker and homogenized in a blender. The YCT mixture was stored in a freezer or refrigerator until use. Refrigerated YCT was used within two weeks of storage.

2.6 Test Procedures

The following sections describe the procedures used for the site sediment and reference toxicity tests.

2.6.1 Site Sediment Toxicity Test

Sediment samples were collected from six locations along the Animas River (A56, A68, A72, A73B, 75B, and Baker Bridge [Bbridge]), one location on Mineral Creek (M34), and one location on Cement Creek (CC49). The two reference locations consisted of A56 and A68. Testing was also performed on negative and positive control sediment for quality assurance purposes. The positive control was spiked with 1000 mg/kg zinc solution intended to substantially reduce survival and growth while the negative control was not spiked and was used to test the overall health of the organisms. The control sediment consisted of sediment collected from Horsecreek Reservoir, located 5 miles south of Hudson, Colorado.

Eight replicates for each site and reference location were tested as well as for the test controls. An initial weight for the *H. azteca* was obtained at the start of the test to verify that the control organisms showed measurable growth after 10 days of exposure (See **Table 2.6-1**). The test chambers, which consisted of 300-mL beakers filled with 100 mL of sediment and 175 mL of overlying MHRW, were placed in a water bath to maintain a constant temperature during the test.

The testing took place over a 10-day period. The quality of the overlying MHRW was measured daily for DO and temperature. Overlying water was measured for hardness, conductivity, ammonia and pH (**Appendix A**) at the start (Day 0) and end (Day 9) of the test. Alkalinity was not measured due to water volume constraints. The amphipods were fed 1 mL of YCT per test chamber per day. The surviving organisms were removed (or “picked”) from the sediment using pipettes, a sieve, and/or Nitex screen at the end of the 10-day test period. Personnel involved with picking organisms from the sediment were first required to show proficiency by retrieving at least 90% of organisms placed into “practice” sediment.

2.6.2 Reference Toxicity Test

The 96-hour reference toxicity test followed procedures outlined in EPA Method 100.1 (EPA, 2000) and was carried out concurrently with the site sediment toxicity test. The test chambers consisted of 200-mL beakers, filled with 100 mL of MHRW, and contained Nitex screen at the bottom as an artificial substrate. MHRW was spiked with zinc sulfate

heptahydrate (ZnSO_4) using a serial dilution approach. ZnSO_4 concentrations were reduced by 50% starting with the highest concentration (referred to as 100%) until the lowest dilution (6.25%) was reached. The following values provide the dilutions and average zinc concentrations (taken from the initial and final measured dissolved Zn results): 100% concentration of ZnSO_4 was 857.5 $\mu\text{g/L}$, followed by 50% (477 $\mu\text{g/L}$), 25% (250 $\mu\text{g/L}$), 12.5% (119.5 $\mu\text{g/L}$), 6.25% (59.55 $\mu\text{g/L}$) and 0% (5 $\mu\text{g/L}$). The zinc concentration used for 0% is one half the laboratory detection limit of 10 $\mu\text{g/L}$. Zinc concentrations were verified using EPA Method 200.7/200.8 and are included in **Table 3.1-2**. Survival and growth were the endpoints for the reference test.

2.7 Pore Water Collection Procedures

Pore water was collected from each individual test chamber for analysis at the start of the test. These samples were collected by placing a push-point pore water sampling probe in each test chamber and extracting the water from within the bedded sediment using a 50 mL syringe. The syringe was then fit with a 0.45 micrometer filter, and the sample was transferred to a 10 mL sample container. The sample was labeled, preserved, and stored at 4°C in the Region 8 Laboratory. The initial pore water samples were analyzed for dissolved metals using EPA Method 200.7 (EPA 1994a) and 200.8 (EPA, 1994b). Pore water samples were not collected at the end of the test due to the risk of extracting *H.azteca* from the sediment during the pore water collection.

2.8 Overlying Surface Water Collection Procedure

Samples for overlying water were collected using treatment group dedicated 60 mL syringes and water was extracted from just below the surface in each replicate for a composite sample. Composite samples were collected for total recoverable and dissolved metals samples and a discrete water sample was collected from each replicate for all ammonia samples. After 50 or 60 mL of water was pulled into the syringe a visual observation of the water was made to ensure that no organisms were inadvertently captured during this process.

3.0 RESULTS

This section presents the results for the site sediment and reference toxicity tests and addresses any issues or unforeseen conditions encountered during the test.

3.1 Site Sediment Toxicity Testing

Sediment, pore water, and overlying surface water samples were analyzed for total recoverable metals (sediment & overlying water) and dissolved metals (pore water & overlying water) using EPA Method 200.7/200.8. **Tables 3.1-1** through **3.1-4** provide the results of these analyses.

The conditions in the test chambers generally met the performance criteria (see **Table 2.0-1**). Daily water chemistry is provided in **Appendix A**. The replicates' variability in hardness met performance criteria of 50% at all sample locations except Control N which had an initial hardness of 386 and a final hardness of 127. Alkalinity was not measured. DO was maintained above the performance criterion of 2.5 mg/L throughout the test. The

overlying water temperatures did not deviate more than $\pm 2^{\circ}\text{C}$ from 23°C , ranging between 21.0°C and 23.1°C during the 10-day test period.

A discrete sample of the overlying water was obtained from each replicate on Day 0 and Day 9 of the test for ammonia analysis using EPA Method 350.1 (EPA 1993). Ammonia ($\text{NH}_3\text{-N}$) concentrations on Day 0 (initial water chemistry) ranged from 0.0053 mg/L in Control-P-04 to 5.916 mg/L $\text{NH}_3\text{-N}$ in A68-01. On Day 9 (final water chemistry) concentrations ranged from 0.0230 mg/L $\text{NH}_3\text{-N}$ in Control P-05 to 1.59 mg/L $\text{NH}_3\text{-N}$ in A68-01 (see **Table 3.1-5**). The average Day 0 and Day 9 ammonia levels measured in the eight replicates of each of the sediment samples used in the toxicity test were compared to pH-dependent acute ammonia criteria. As shown in **Table 3.1-5**, all ammonia levels fell below their respective acute or chronic ammonia criteria. The ammonia criteria were calculated using the “salmonids present” equation, which is provided on p. 54 of the Colorado Department of Public Health and Environment Water Quality Control Commission (CDPHE): Regulation No. 31 (2012).

Surviving *H. azteca* were collected at the end of the 10 day test from each test chamber, counted, placed in aluminum weigh boats, and dried for at least 24 hrs at 80°C . Every effort was made to ensure that sediment particles were not inadvertently added to the weigh boats with the organisms. Pans with dried *H. azteca* were then weighed. All information was recorded on laboratory bench sheets.

Comprehensive Environmental Toxicity Information System (CETIS) statistical software (2011) was used to establish the significance differences between *H. azteca* survival and biomass between groups after 10 days of exposure in the sediment samples (see **Attachment 1**). **Figure 3.1-1** presents the results for survival and **Figure 3.1-2** presents the results for biomass.

Survival Results

Survival results for each replicate and average per location are included in the CETIS worksheets (**Attachment 1**). Control P and Control N both showed an average of 97.5% survival and were therefore indistinguishable from each other. Control N met the minimum performance criterion of $>80\%$ survival. Control P had unexpectedly high survival but did not influence the outcome of the toxicity test. Site sample results were only compared to Control N to determine significant survival and growth. The available analytical data (see **Tables 3.1-1 to 3.1-4**) show that the Zn levels in Control P were consistently similar to those measured in Control N. An error occurred with the spiking procedure or possibly zinc was washed out of the sandy substrate during the daily water exchange procedures.

Reference locations A56 and A68 showed only $62.5 \pm 8.2\%$ and $56.3 \pm 3.5\%$ survival, respectively. Location A72 had $36.3 \pm 4.2\%$ survival, location A73B had $5 \pm 1.9\%$ survival, location A75B had $48.8 \pm 5.2\%$ survival and location M34 had $8.8 \pm 3.5\%$ survival. None of the organisms survived at location CC49, whereas location Bbridge showed $76.3 \pm 3.75\%$ survival.

Biomass Results

Biomass results for each replicate and average per location are included in the CETIS worksheets (**Attachment 1**). Average biomass for each location was calculated by dividing the total weight of all surviving *H. azteca* per sample location by the total number of *H. azteca* introduced per sample location on Day 0 of the test (i.e. [total weight of *H. azteca* from all eight replicates per sample location] / [8 replicates x 10 *H. azteca* introduced for each sample location]). The results show that Control N had an average biomass of 69.8 ± 3.5 $\mu\text{g}/\text{organism}$. Reference sample locations A56 and A68 had an average biomass of 20.3 ± 1.9 $\mu\text{g}/\text{organism}$ and 22.6 ± 1.6 $\mu\text{g}/\text{organism}$, respectively. The following values represent the average biomass results for the remaining sample locations: A72 (16.1 ± 1.7 $\mu\text{g}/\text{organism}$), A73B (4.0 ± 1.7 $\mu\text{g}/\text{organism}$), 75B (17.8 ± 1.9 $\mu\text{g}/\text{organism}$), M34 (5.1 ± 2 $\mu\text{g}/\text{organism}$), and Bbridge (26.2 ± 1 $\mu\text{g}/\text{organism}$). Sample location CC49 had zero biomass because none of the *H. azteca* survived.

Growth Results

Growth for Control N was analyzed in order to determine if the Test Acceptability Criteria (TAC) of a measurable increase in growth between the start (Day 0) and the end (Day 10) of the test was met. All Control N growth results, along with supplemental growth results for Control P and each sample location are included in **Table 3.1-7**. The mean weight per survivor in each replicate is presented in Table 3.1-7. The average growth was analyzed for each location with the formula: ([total weight of *H. azteca* from all eight replicates per sample location] / [total surviving *H. azteca* for each sample location at the end of the test]) – initial weight). The initial average organism weight was 22.9 $\mu\text{g}/\text{organism}$ (**Table 2.6-1**), whereas the final average organism weight for Control N was 71.7 $\mu\text{g}/\text{organism}$ (i.e., +48.63 $\mu\text{g}/\text{organism}$ or 212% growth increase). The surviving organisms exposed to reference samples A56 showed an +11.51 $\mu\text{g}/\text{organism}$ increase (50.17% growth increase) and A68 showed a +17.18 $\mu\text{g}/\text{organism}$ increase (74.9% growth increase).

3.2 Reference Toxicity Test

Overlying water quality parameters were consistent throughout the 96-hour reference toxicity test (see **Appendix B**). The performance criterion for EPA Method 100.1 requires no more than 50% change for alkalinity and hardness, whereas DO must be maintained above 2.5 mg/L. Test chamber temperatures ranged between 21.2°C and 22.7°C during the test period. The variability in hardness was less than 50% within each test chamber, and DO levels ranged between 5.65 mg/L and 7.72 mg/L. Alkalinity was not measured due to water volume constraints.

CETIS used the Trimmed Spearman-Kärber method to calculate the EC₅₀ 143 $\mu\text{g}/\text{L}$ zinc with an UCL of 161 and an LCL of 127 $\mu\text{g}/\text{L}$ zinc. These values correspond with historical LC50 values calculated from reference toxicity tests performed at the Region 8 Laboratory. Figure 3.2-2 shows the acute reference toxicant control chart for *H. azteca* exposed to zinc.

Survival Results

The surviving organisms were collected at the end of the 96-hour reference toxicity test and counted. **Figure 3.2-1** provides the results. The control (5 µg/L Zn) passed the performance criterion of > 80% survival, with average survival of 100%. The following values show the average zinc concentrations and % survivals from the reference toxicity test: 59.55 µg/L zinc = 95% survival, 119.5 µg/L zinc = 72.5% survival, 250 µg/L zinc = 2.5% survival, 477 µg/L zinc = 0% survival and 857.5 µg/L zinc = 0% survival. A 96-hour LC₅₀ of 143 µg/L was calculated using the Trimmed Spearman-Kärber Estimates (see **Attachment 2**). *Note that CETIS uses the term “EC50” (Median Effective Concentration effecting 50% of the test organisms) instead of LC₅₀.*

A discrete sample of the overlying water was obtained from each replicate on Day 0 and Day 4 of the test for ammonia analysis using EPA Method 350.1 (EPA 1993). Ammonia (NH₃-N) levels on Day 0 (initial water chemistry) ranged from 0.00452 mg/L in the 50%-03 replicate to 0.01380 mg/L NH₃-N in the 100%-04 replicate. On Day 4 (final water chemistry), the ammonia levels ranged from 0.0159 mg/L NH₃-N in the 100%-02 replicate to 0.3176 mg/L NH₃-N in the 12.5%-01 replicate. **Table 3.1-6** provides the ammonia data. The average Day 0 and Day 4 ammonia levels measured in the four replicates of each of the samples used in the reference toxicity test were compared to pH-dependent acute ammonia criteria. None of the measured ammonia levels exceeded their respective criteria. Note that the acute ammonia criteria were calculated using the “salmonids present” equation, provided on p. 54 of the CDPHE (2012).

4.0 DISCUSSION

The survival and biomass results of the Animas River sediment toxicity test were compared to the two reference samples (A56 and A68) as well as the negative control. Below is a discussion of the results.

Survival

The CETIS software was used to perform a Dunnett’s Multiple Comparison Test when comparing Control N, A56, and A68 to site samples to determine the significance ($p \leq 0.05$) of the observed survival after 10 days of exposure (**Attachment 1**). Reference locations A56 and A68 had 62.5% and 56.25% survival respectively. Sample locations A75B (48.75% survival) and Bbridge (76.25% survival) were found not to be statistically different when compared to either of the reference locations. The survival for A72 (36.25%), A73B (5%), M34 (8.75%), and CC49 (0%) were all statistically different when compared to both reference locations. Note that one replicate in Control N had 11 *H. azteca* at test termination. The results were entered into CETIS to include 11 organisms exposed for that replicate representing 100% survival.

All Site samples (A72, A73B, A75B, M34 and CC49) were statistically different when compared to Control N. Control N (together with Control P) had the highest survival of all samples in the test.

Biomass

CETIS was used to perform a Dunnett Multiple Comparison Test (for reference locations comparisons against Site samples) and Steel Many-One Rank Test (for Control N comparison against Site samples) to determine significant ($p \leq 0.05$) difference in observed biomass after 10 days of exposure (**Attachment 1**). Reference locations had a mean biomass of $20.3 \pm 1.9 \mu\text{g}$ (A56) and $22.6 \pm 1.6 \mu\text{g}$ (A68). When reference location A56 was compared to Site samples, locations A73B, M34, and CC49 were significantly different. When reference location A68 was compared to Site samples, locations A72, A73B, M34 and CC49 were significantly lower.

Biomass for all Site samples (A72, A73B, A75B, M34, CC49, and Bbridge) was statistically lower when compared to the Control N biomass value of $69.8 \pm 3.5 \mu\text{g}$.

Growth

Control N passed TAC with measurable growth. The final average weight per organism was $71.7 \mu\text{g}$. This value represented a growth increase of about $48.63 \mu\text{g/organism}$ when compared to the initial average weight of *H. azteca* ($22.94 \mu\text{g/organism}$).

Results consistently show that survival and biomass at locations A73B, M34, and CC49 are significantly impacted when compared to the two reference locations. Sample location A72 was determined to be significantly impacted for survival when compared to both reference locations. Biomass for sample location A72 was significantly impacted when compared with reference location A68 but not with reference location A56. Survival and biomass in all Site samples were significantly affected when compared to Control N. Reference locations were not compared to Control N.

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Tables

Table 2.0-1 Test Conditions and Acceptability Criteria for 10 Day Sediment Toxicity Testing Using *H. azteca*

Parameter	Conditions
Test Type	Whole sediment toxicity test with renewal of overlying water
Test Duration	10 days
Temperature	Daily Mean Test Temperature 23 +/- 1°C; Instantaneous temperature 23 +/- 3°C
Light Quality	Wide spectrum fluorescent lights
Illuminance	100 to 1000 lux
Photoperiod	16 light 8 dark
Test Chamber	300 mL beaker
Sediment Volume	100 mL
Overlying Water Volume	175 mL
Renewal of Overlying Water	2 volumes per day
Age of Organisms	7-14 day old at start of test
Number of Organisms/chamber	10
Number of Replicates	8 for whole sediment; 4 for reference test
Feeding	YCT food fed 1.0 mL per day to each test chamber
Aeration	None
Overlying Water	Moderately Hard Reconstituted Water
Test Chamber Cleaning	Clean screens if clogged
Overlying Water Quality	Initial and final measurements of: hardness, alkalinity, conductivity, pH, ammonia (hardness, alkalinity and ammonia should not vary more than 50%) Daily measurements of: temperature and dissolved oxygen
Endpoints	Survival and growth
Test Acceptability	Minimum mean control survival of 80% and measurable growth of test organisms in the control sediment

Table 2.5-1 Initial Feed Weight Data Sheets
 December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
 10-Day Static Renewal
 YCT Dry Weight

Feed Dry Weight Rep #1		
Weigh boat	1.5406	g
Weigh boat + 1 mL wet feed	2.5935	g
1 mL wet feed	1.0529	g
Weight boat + dry feed	1.5432	g
Dry feed	0.0026	g/mL
Dry feed	2.6	g/L

Feed Dry Weight Rep #2		
Weigh boat	1.5271	g
Weigh boat + 1 mL wet feed	2.5548	g
1 mL wet feed	1.0277	g
Weight boat + dry feed	1.53	g
Dry feed	0.0029	g/mL
Dry feed	2.9	g/L

Feed Dry Weight Rep#3		
Weigh boat	1.542	g
Weigh boat + 1 mL wet feed	2.515	g
1 mL wet feed	0.973	g
Weight boat + dry feed	1.5446	g
Dry feed	0.0026	g/mL
Dry feed	2.6	g/L

Table 2.6-1 Initial *H. azteca* Weight Data Sheet
December 2012 Animas River Sediment Toxicity Test
10-Day Static Renewal

Initial Dry Weight: 80 Organisms	
Weigh Boat (empty)	208543.1 µg
Weigh Boat with 80 organisms (dried)	210378.4 µg
Average Organism	22.94 µg

Table 3.1-1

December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
Initial Pore Water Dissolved Metals Results (µg/L)

STATION_ID	Aluminum	Antimony	Arsenic	Barium	Beryllium	Cadmium	Calcium	Chromium	Cobalt	Copper	Hardness	Iron	Lead	Magnesium	Manganese	Nickel	Potassium	Selenium	Silver	Sodium	Strontium	Thallium	Vanadium	Zinc
Control-P	232	<0.500U	1.14J	26.3	<2.00U	<0.100U	28700	<1.00U	<0.100U	26.4	118	<100U	0.428	12000	4870	0.663J	3910	0.659J	<0.500U	27000	285	<0.500U	<2.00U	33.5
Control-N	32.8J	1.29	5.68	27.1	<2.00U	<0.100U	309000	<1.00U	4.58	12.8	98	<100U	0.203	10300	6180	1.96	4480	<0.500U	<0.500U	24100	289	<0.500U	<2.00U	29.0
A56	57.1	1.71	1.93J	66.2	<2.00U	0.124J	27500	<1.00U	0.362	15.1	182	<100U	8.13	15100	9290	1.62	3130	0.775J	<0.500U	29200	453	<0.500U	<2.00U	27.5
A68	146	<0.500U	3.77	121	<2.00U	0.123J	22200	<1.00U	0.163J	25.6	120	<100U	13.3	13500	14500	1.27	3290	1.13	<0.500U	25300	235	<0.500U	<2.00U	27.6
A72	27.6J	<0.500U	<0.500U	28.3	<2.00U	0.931	47900	<1.00U	0.916	12.1	143	<100U	0.161J	15100	9630	0.662J	5000	1.57	<0.500U	28100	301	<0.500U	<2.00U	26.1
A73B	48.2J	<0.500U	<0.500U	32.4	<2.00U	0.213	25700	<1.00U	3.71	9.54	133	224J	<0.100U	14400	7320	0.545J	4130	0.977J	<0.500U	27700	285	<0.500U	<2.00U	38.0
A75B	47.8J	<0.500U	1.14J	33.2	<2.00U	<0.100U	32500	<1.00U	3.12	5.45	161	820	0.165J	15300	6180	5.67	2790	0.879J	<0.500U	26300	405	<0.500U	<2.00U	516
M-34	38.8J	<0.500U	0.559J	28.5	<2.00U	0.412	36200	<1.00U	14.6	6.06	1150	3680	0.168J	91700	1670	10.5	18500	10.2	<0.500U	267000	1720	<0.500U	<2.00U	13.1J
CC-49	1120	<0.500U	<0.500U	12.9	<2.00U	1.91	39000	<1.00U	31.5	26.4	107	2120	14.5	8670	3.57J	2.20	4120	1.53	<0.500U	26100	217	<0.500U	3.31	13.4J
Bbridge	79.3	<0.500U	0.802J	49.3	<2.00U	<0.100U	29700	<1.00U	2.35	5.93	158	169J	0.140J	16500	8920	2.68	3370	1.53	<0.500U	28200	308	<0.500U	<2.00U	37.9

Qualifiers:

J = estimated

U = non-detect

Table 3.1-4

December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
 Initial Sediment Total Recoverable Metals Results

STATION_ID	ANALYSIS	UNITS	Aluminum	Barium	Beryllium	Calcium	Copper	Iron	Lead	Magnesium	Manganese	Potassium	Sodium	Strontium	Zinc
Control-P	ICPOE Tot. Rec. Metals	mg/kg dry wt	1300D	16.2D	<1.99U	930D	2.19D	2720D	<9.94U	434D	34.3D	347JD	<249U	8.44JD	21.6D
Control-N	ICPOE Tot. Rec. Metals	mg/kg dry wt	1340D	28.7D	<1.96U	2770D	2.78D	2860D	<9.81U	610D	76.6D	420JD	337JD	18.6D	19.8D
A56	ICPOE Tot. Rec. Metals	mg/kg dry wt	9790D	113D	2.76JD	4430D	306D	28700D	2070D	4630D	6020D	900JD	<255U	44.9D	3530D
A68	ICPOE Tot. Rec. Metals	mg/kg dry wt	14500D	190D	5.14D	5330D	605D	43900D	2600D	5040D	12100D	1200D	<250U	75.6D	7630D
A72	ICPOE Tot. Rec. Metals	mg/kg dry wt	24800D	195D	<2.01U	4270D	198D	60600D	704D	6570D	4320D	1420D	<252U	87.9D	968D
A73B	ICPOE Tot. Rec. Metals	mg/kg dry wt	17200D	103D	<1.99U	2880D	232D	48500D	557D	4040D	4430D	567JD	<249U	42.4D	1240D
A75B	ICPOE Tot. Rec. Metals	mg/kg dry wt	47400D	145D	5.63D	6320D	415D	81400D	436D	3980D	4440D	1360D	<251U	99.9D	4980D
M-34	ICPOE Tot. Rec. Metals	mg/kg dry wt	32800D	105D	<1.96U	4290D	91.4D	62300D	152D	5340D	1220D	935JD	<245U	61.4D	323D
CC-49	ICPOE Tot. Rec. Metals	mg/kg dry wt	4140D	55.7D	<1.96U	1200D	57.8D	289000D	206D	1720D	307D	450JD	<245U	17.8D	132D
Bbridge	ICPOE Tot. Rec. Metals	mg/kg dry wt	44800D	206D	5.72D	7890D	377D	78500D	471D	4330D	8790D	1350D	<252U	123D	9060D

STATION_ID	ANALYSIS	UNITS	Antimony	Arsenic	Cadmium	Chromium	Cobalt	Nickel	Selenium	Silver	Thallium	Vanadium
Control-P	ICPMS Tot. Rec. Metals	ug/kg dry wt	<497U	745JD	<99.4U	2830D	1160D	2070D	<497U	<497U	<497U	2170JD
Control-N	ICPMS Tot. Rec. Metals	ug/kg dry wt	<490U	<490U	133JBD	2890D	1790D	2110D	<490U	<490U	<490U	2160JD
A56	ICPMS Tot. Rec. Metals	ug/kg dry wt	9530D	79600D	9220BD	5480D	13100D	8580D	<510U	10300D	<510U	14300D
A68	ICPMS Tot. Rec. Metals	ug/kg dry wt	9340D	82400D	16700BD	8800D	15400D	15200D	1220D	12000D	1160D	18300D
A72	ICPMS Tot. Rec. Metals	ug/kg dry wt	1790D	45600D	3280BD	4660D	21800D	7180D	<503U	3530D	688JD	23200D
A73B	ICPMS Tot. Rec. Metals	ug/kg dry wt	2270D	29100D	5220BD	4700D	21200D	11500D	<498U	3290D	<498U	17300D
A75B	ICPMS Tot. Rec. Metals	ug/kg dry wt	2060D	37500D	10300BD	5420D	29900D	16100D	1110D	2070D	540JD	22900D
M-34	ICPMS Tot. Rec. Metals	ug/kg dry wt	<491U	21000D	1060BD	3640D	14800D	4520D	<491U	558JD	<491U	20500D
CC-49	ICPMS Tot. Rec. Metals	ug/kg dry wt	1510D	66700D	338BD	4710D	2520D	1890D	<490U	1200D	<490U	56200D
Bbridge	ICPMS Tot. Rec. Metals	ug/kg dry wt	2110D	40200D	16900BD	5260D	60800D	31000D	<503U	2200D	547JD	24200D

Qualifiers:

D = diluted sample

J = estimated

U = non-detect

Table 3.1-5: Initial and Final Average Ammonia Results for December 2012 Upper Animas River Sediment Toxicity Test Using *H. azteca*

Replicate ID	Initial Measured Ammonia Conc. (mg N/L)	Initial Measured pH	Initial Average Measured Ammonia Conc. (mg N/L)	Initial Average Measured pH	Initial Ammonia Criterion (mg N/L) ^a	Final Measured Ammonia Conc. (mg N/L) ^b	Final Measured pH ^b	Final Average Measured Ammonia Conc. (mg N/L)	Final Average Measured pH	Final Ammonia Criterion (mg N/L) ^a
Control-P-01	0.0230	7.4	0.0085	7.45	14.40	0.0384	6.7	0.0405	6.74	29.08
Control-P-02	0.0061	7.4				0.0547	6.7			
Control-P-03	0.0069	7.5				0.0544	6.7			
Control-P-04	0.0053	7.5				0.0408	6.7			
Control-P-05	0.0062	7.4				0.0230	6.7			
Control-P-06	0.0063	7.5				0.0301	6.8			
Control-P-07	0.0088	7.4				0.0323	6.8			
Control-P-08	0.0054	7.5				0.0501	6.8			
Control-N-01	1.2810	6.9	1.4109	6.97	24.65	0.4570	5.8	0.4732	6.23	35.29
Control-N-02	1.5980	6.9				0.4690	6.0			
Control-N-03	1.3630	7.0				0.5375	6.1			
Control-N-04	1.3190	6.9				0.4327	6.2			
Control-N-05	1.5660	6.9				0.5155	6.4			
Control-N-06	1.2860	7.1				0.3950	6.4			
Control-N-07	1.5280	7.0				0.5453	6.5			
Control-N-08	1.3460	7.1				0.4339	6.5			
A56-01	1.7380	7.1	1.6259	7.24	18.78	0.0422	7.1	0.0427	7.11	21.67
A56-02	1.9840	7.2				0.0450	7.1			
A56-03	1.2580	7.2				0.0340	7.1			
A56-04	1.7850	7.2				0.0375	7.1			
A56-05	1.3480	7.3				0.0463	7.1			
A56-06	1.5650	7.3				0.0376	7.1			
A56-07	1.6000	7.3				0.0528	7.1			
A56-08	1.7290	7.3				0.0465	7.2			
A68-01	5.9160	7.4	5.0698	7.51	13.06	1.5900	7.2	1.2675	7.22	19.25
A68-02	5.0510	7.5				1.1350	7.2			
A68-03	5.0770	7.5				1.1840	7.2			
A68-04	4.8800	7.5				1.5560	7.2			
A68-05	5.0990	7.5				1.2010	7.2			
A68-06	4.5280	7.6				1.1580	7.3			
A68-07	5.1330	7.6				1.2630	7.2			
A68-08	4.8740	7.6				1.0530	7.3			
A72-01	0.2228	7.1	0.2050	7.08	22.44	0.0654	7.0	0.1057	7.01	23.87
A72-02	0.1624	7.1				0.0584	7.0			
A72-03	0.2154	7.1				0.2117	7.0			
A72-04	0.2423	7.1				0.1607	7.0			
A72-05	0.2230	7.1				0.1321	7.0			
A72-06	0.1938	7.1				0.0595	7.0			
A72-07	0.1964	7.0				0.0979	7.0			
A72-08	0.1840	7.1				0.0603	7.0			
A73B-01	1.0890	7.5	0.8890	7.38	15.71	0.0664	7.2	0.0416	7.15	20.76
A73B-02	0.6481	7.4				0.0455	7.2			
A73B-03	0.9262	7.4				0.0338	7.2			
A73B-04	0.8286	7.4				0.0344	7.2			
A73B-05	0.9758	7.4				0.0421	7.1			
A73B-06	0.8755	7.3				0.0399	7.1			
A73B-07	0.9011	7.3				0.0346	7.1			
A73B-08	0.8677	7.3				0.0364	7.1			
A75B-01	1.8390	7.0	1.7875	7.06	22.87	0.0432	7.0	0.0521	6.99	24.23
A75B-02	1.8770	7.0				0.0935	7.0			
A75B-03	1.7820	7.0				0.0393	7.0			
A75B-04	1.5890	7.1				0.0537	7.0			
A75B-05	1.9480	7.1				0.0457	7.0			
A75B-06	1.8490	7.1				0.0415	7.0			
A75B-07	1.7990	7.1				0.0450	7.0			
A75B-08	1.6170	7.1				0.0545	7.0			
M34-01	0.1279	6.2	0.1313	6.27	34.95	0.3956	6.2	0.4178	6.48	32.86
M34-02	0.1294	6.2				0.3824	6.3			
M34-03	0.1302	6.2				0.3438	6.5			
M34-04	0.1424	6.3				0.4928	6.5			
M34-05	0.1385	6.3				0.4676	6.6			
M34-06	0.1294	6.3				0.4916	6.6			
M34-07	0.1335	6.3				0.4146	6.6			
M34-08	0.1187	6.4				0.3536	6.6			
CC49-01	0.1200	7.3	0.1009	6.47	32.98	0.3776	7.1	0.4138	5.09	38.70
CC49-02	0.1235	7.2				0.4361	5.4			
CC49-03	0.1396	6.7				0.4105	4.3			
CC49-04	0.0770	6.4				0.3095	4.9			
CC49-05	0.0956	6.3				0.4655	4.2			
CC49-06	0.0900	6.1				0.5554	4.8			
CC49-07	0.0856	5.9				0.4005	4.8			
CC49-08	0.0756	5.9				0.3551	5.2			
Bbridge-01	2.3670	6.5	2.1519	6.85	27.21	0.0411	6.8	0.0415	6.89	18.31
Bbridge-02	1.6270	6.7				0.0387	6.9			
Bbridge-03	2.3920	6.8				0.0468	6.8			
Bbridge-04	2.1860	6.8				0.0418	6.9			
Bbridge-05	2.3110	6.9				0.0420	6.9			
Bbridge-06	2.2140	7.0				0.0394	6.9			
Bbridge-07	2.0910	7.0				0.0441	7.0			
Bbridge-08	2.0270	7.0				0.0379	7.0			

^a The sample-specific acute ammonia criterion was calculated using the "salmon present" formula on p. 54 of the Colorado Department of Public Health and Environment, Water Quality Control Commission, Regulation No. 31: The Basic Standards and Methodologies for Surface Water (5 CCR 1002-31).

^b Values shown are either the measurements made at the end of the test (day 10) or earlier if all test organisms died before the 10-day exposure period was completed.

Table 3.1-6 Initial and Final Average Ammonia Results for December 2012 Upper Animas River Concurrent Reference Toxicity Test Using *H. azteca*

Replicate ID	Initial Measured Ammonia Conc. (mg N/L) ^a	Initial Measured pH	Initial Average Measured Ammonia Conc. (mg N/L)	Initial Average Measured pH	Initial Ammonia Criterion (mg N/L) ^a	Final Measured Ammonia Conc. (mg N/L)	Final Measured pH	Final Average Measured Ammonia Conc. (mg N/L)	Final Average Measured pH	Final Ammonia Criterion (mg N/L) ^a
Ref Control-01	0.00543	7.16	0.0061	7.23	19.17	0.2191	7.35	0.1850	7.16	20.67
Ref Control-02	0.00575	7.20				0.1978	7.38			
Ref Control-03	0.00559	7.25				0.1799	6.84			
Ref Control-04	0.00755	7.29				0.1431	7.06			
6.25%-01	0.00494	7.34	0.0052	7.38	15.77	0.1444	7.00	0.1198	7.05	23.14
6.25%-02	0.00577	7.36				0.1128	7.02			
6.25%-03	0.00533	7.39				0.1045	7.06			
6.25%-04	0.00481	7.43				0.1174	7.10			
12.5%-01	0.00865	7.45	0.0072	7.48	13.63	0.3176	7.14	0.1735	7.20	19.67
12.5%-02	0.00947	7.46				0.1355	7.18			
12.5%-03	0.00519	7.49				0.1141	7.23			
12.5%-04	0.00541	7.53				0.1267	7.26			
25%-01	0.00471	7.53	0.0053	7.54	12.45	0.0949	--	0.0998	7.29	17.73
25%-02	0.00501	7.53				0.0842	--			
25%-03	0.00598	7.54				0.0792	--			
25%-04	0.00560	7.57				0.1408	7.29			
50%-01	0.00568	7.54	0.0050	7.54	12.50	0.0505	--	0.0384	NC	NA
50%-02	0.00510	7.48				0.0313	--			
50%-03	0.00452	7.61				0.0362	--			
50%-04	0.00458	7.53				0.0356	--			
100%-01	0.00493	7.55	0.0075	7.55	12.31	0.0171	--	0.0181	NC	NA
100%-02	0.00471	7.55				0.0159	--			
100%-03	0.00665	7.50				0.0198	--			
100%-04	0.01380	7.60				0.0194	--			

NA = Not available
 NC = Not calculated

^a The sample-specific acute ammonia criterion was calculated using the "salmon present" formula on p. 54 of the Colorado Department of Public Health and Environment, Water Quality Control Commission, Regulation No. 31: The Basic Standards and Methodologies for Surface Water (5 CCR 1002-31).

^b Values shown are either the measurements made at the end of the test (day 4) or earlier if all test organisms died before the 4-day exposure period was completed.

Prepared by: EC 7/19/13
 Reviewed by: BGK 7/23/13

Table 3.1-7 December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
Weight Data Sheets: 10-Day Static Renewal

Start Date	12/10/12	Drying Time	24 hours
End Date	12/20/12	Oven Temp (°C)	70°C
Weighing Date	01/09/13	Organism	<i>H. azteca</i>
No. of Replicates	8	Initial Weight (µg)	22.94
Feed Rate/Type	YCT/Daily	Analysts	SA,LC,CL

Replicate I.D.	Weight of Oven Dried Pan (µg)	Pan + Dried Organisms (µg)	Dry Organisms (µg)	Number of Survivors	Mean Weight per Survivor (µg)	Sample Mean (µg)
Control-P-01	207294.6	207694	399.4	10	39.94	41.48
Control-P-02	207493.7	207886.2	392.5	10	39.25	
Control-P-03	207230.1	207778.4	548.3	10	54.83	
Control-P-04	206113.8	206463.3	349.5	9	38.83	
Control-P-05	209306.2	209611	304.8	10	30.48	
Control-P-06	208216.3	208784.9	568.6	10	56.86	
Control-P-07	208942.2	209243.6	301.4	10	30.14	
Control-P-08	205478.8	206013.1	534.3	9	59.37	
Control-N-01	207119	207723	604	10	60.40	71.57
Control-N-02	206639.5	207242.9	603.4	9	67.04	
Control-N-03	209331.6	210156.3	824.7	11	74.97	
Control-N-04	206219.7	207130.9	911.2	10	91.12	
Control-N-05	207477.6	208092.5	614.9	9	68.32	
Control-N-06	207049.1	207710.5	661.4	10	66.14	
Control-N-07	208251	208904.2	653.2	9	72.58	
Control-N-08	208054	208774.1	720.1	10	72.01	
A56-01	209603.7	209717.5	113.8	3	37.93	34.45
A56-02	209189.1	209456.2	267.1	7	38.16	
A56-03	207747.3	207921.2	173.9	5	34.78	
A56-04	206852.2	207075.9	223.7	8	27.96	
A56-05	209002.3	209244.5	242.2	9	26.91	
A56-06	205403.1	205621.2	218.1	7	31.16	
A56-07	207791.3	207936.4	145.1	3	48.37	
A56-08	208463.9	208706.5	242.6	8	30.33	
A68-01	208033	208234	201	5	40.20	40.12
A68-02	206546.4	206746.1	199.7	6	33.28	
A68-03	205690	205981.9	291.9	7	41.70	
A68-04	206756.1	206977.3	221.2	6	36.87	
A68-05	209336.4	209583.3	246.9	6	41.15	
A68-06	207295.6	207443.7	148.1	4	37.03	
A68-07	208476.6	208705.8	229.2	5	45.84	
A68-08	207653.5	207923.0	269.5	6	44.92	
A72-01	207566.1	207684.6	118.5	2	59.25	45.64
A72-02	207038.5	207180.8	142.3	4	35.57	
A72-03	206675.8	206749.4	73.6	2	36.80	
A72-04	206609.1	206778.9	169.8	3	56.60	
A72-05	207490.5	207677.7	187.2	4	46.80	
A72-06	205252.2	205461.6	209.4	5	41.88	
A72-07	204860	205041.0	181	5	36.20	
A72-08	205829.8	206038.0	208.2	4	52.05	

Table 3.1-7 December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
Weight Data Sheets: 10-Day Static Renewal

Start Date	12/10/12	Drying Time	24 hours
End Date	12/20/12	Oven Temp (°C)	70°C
Weighing Date	01/09/13	Organism	<i>H. azteca</i>
No. of Replicates	8	Initial Weight (µg)	22.94
Feed Rate/Type	YCT/Daily	Analysts	SA,LC,CL

Replicate I.D.	Weight of Oven Dried Pan (µg)	Pan + Dried Organisms (µg)	Dry Organisms (µg)	Number of Survivors	Mean Weight per Survivor (µg)	Sample Mean (µg)
A73B-01	208092	--	--	0	--	79.38
A73B-02	205437.5	--	--	0	--	
A73B-03	206460.1	--	--	0	--	
A73B-04	205380.7	205445	64.3	1	64.30	
A73B-05	204644.8	--	--	0	--	
A73B-06	205767.8	205865.9	98.1	1	98.10	
A73B-07	206877.7	206998.2	120.5	1	120.50	
A73B-08	206120.5	206155.1	34.6	1	34.60	
A75B-01	205789.7	205981.8	192.1	6	32.02	37.13
A75B-02	208670.9	208846.4	175.5	6	29.25	
A75B-03	208836.3	208926.6	90.3	3	30.10	
A75B-04	208491.7	208720.8	229.1	5	45.82	
A75B-05	207661.2	207809.1	147.9	5	29.58	
A75B-06	206346.2	206473.2	127	3	42.33	
A75B-07	203809.8	204014.4	204.6	4	51.15	
A75B-08	207994	208251.4	257.4	7	36.77	
M34-01	209594.3	--	--	0	--	114.50
M34-02	206387.3	206533.4	146.1	3	48.70	
M34-03	206176.2	--	--	0	--	
M34-04	207572.2	207686.7	114.5	1	114.50	
M34-05	208422	--	--	0	--	
M34-06	206302.5	206312	9.5	1	9.50	
M34-07	208254.3	208334	79.7	1	79.70	
M34-08	207897.3	207954.3	57	1	57.00	
CC49-01	205074.4	--	--	0	--	#DIV/0!
CC49-02	207328	--	--	0	--	
CC49-03	205978.1	--	--	0	--	
CC49-04	205910.7	--	--	0	--	
CC49-05	204673.6	--	--	0	--	
CC49-06	206851.3	--	--	0	--	
CC49-07	204953.4	--	--	0	--	
CC49-08	207055.2	--	--	0	--	
BBRIDGE-01	205991.7	206249.9	258.2	9	28.69	34.78
BBRIDGE-02	206000.4	206248.9	248.5	8	31.06	
BBRIDGE-03	208297.6	208609.1	311.5	8	38.94	
BBRIDGE-04	205253	205496.6	243.6	8	30.45	
BBRIDGE-05	208943.9	209240.2	296.3	8	37.04	
BBRIDGE-06	208583.4	208820.8	237.4	6	39.57	
BBRIDGE-07	208898	209129.3	231.3	6	38.55	
BBRIDGE-08	208620.5	208892.3	271.8	8	33.97	

Figures

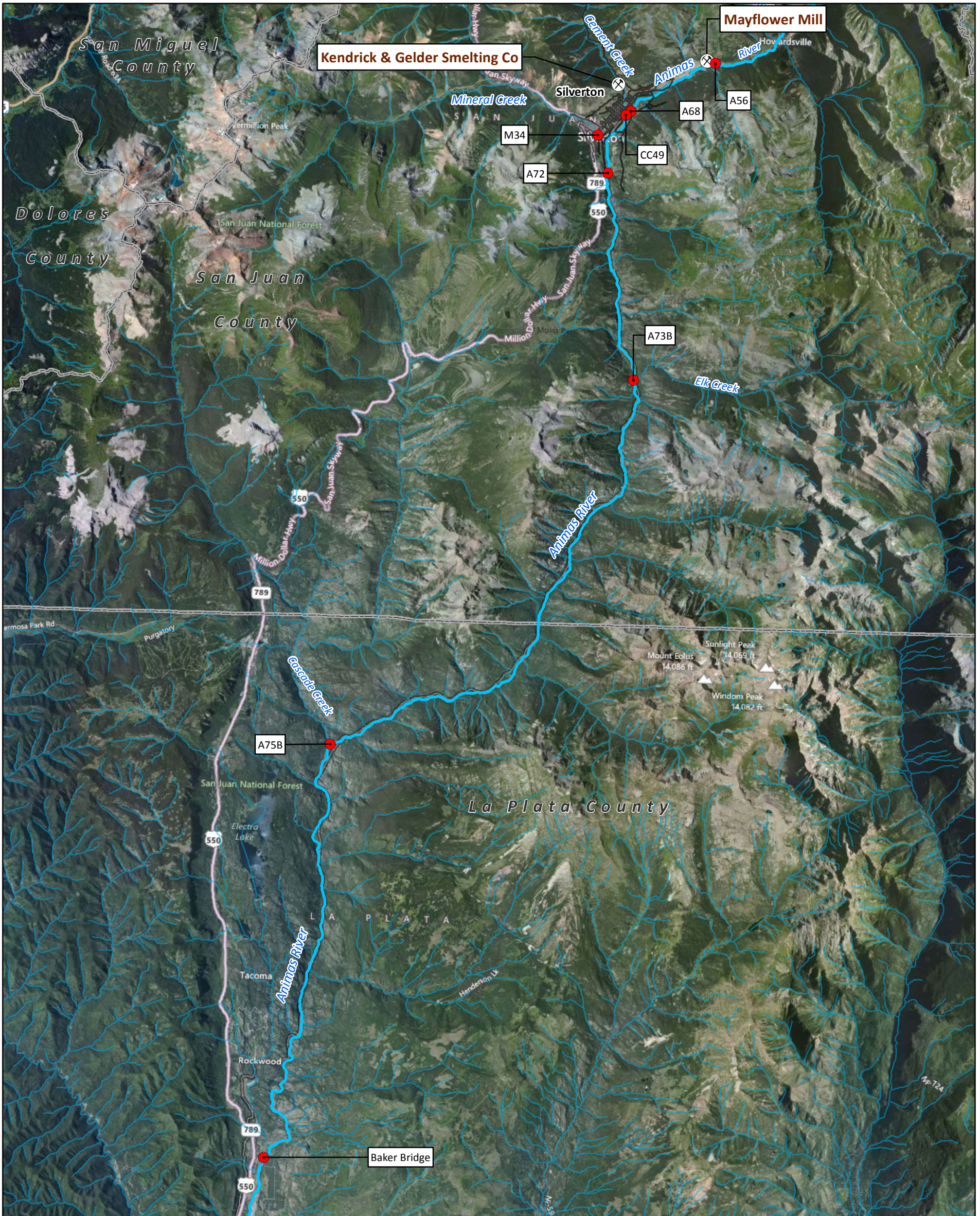


Figure 2.2-1
2012 Upper Animas River
Sediment Toxicity Test Sample Locations

Date: November 12, 2013

Data Sources:

Sample Locations: U.S. EPA Region 8 (2012)
Mine Locations: U.S. EPA and ESAT (2012)
Rivers and Streams: CDOW 1:24k (2004)
County Boundaries: U.S. Census Bureau (2011)
Image: Bing (2013)

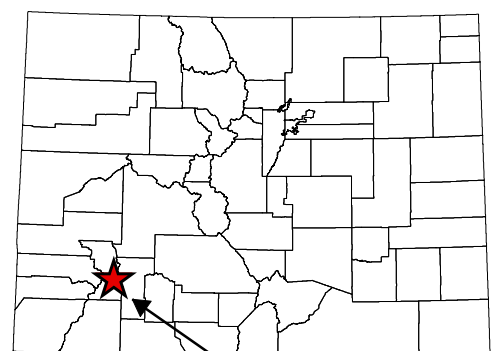
Coordinate System/Projection:
 UTM Zone 13 North, NAD 83, Meters



0 1 2 Miles

0 1 2 Kilometers

Colorado



Area of Interest







-  Sample Locations
-  Mine Locations
-  Rivers and Streams
-  County Boundaries

Figure 3.1-1
2012 Upper Animas River Sediment Toxicity Test Using *H. azteca*
Average Percent Survival + 1 SD per Sampling Location

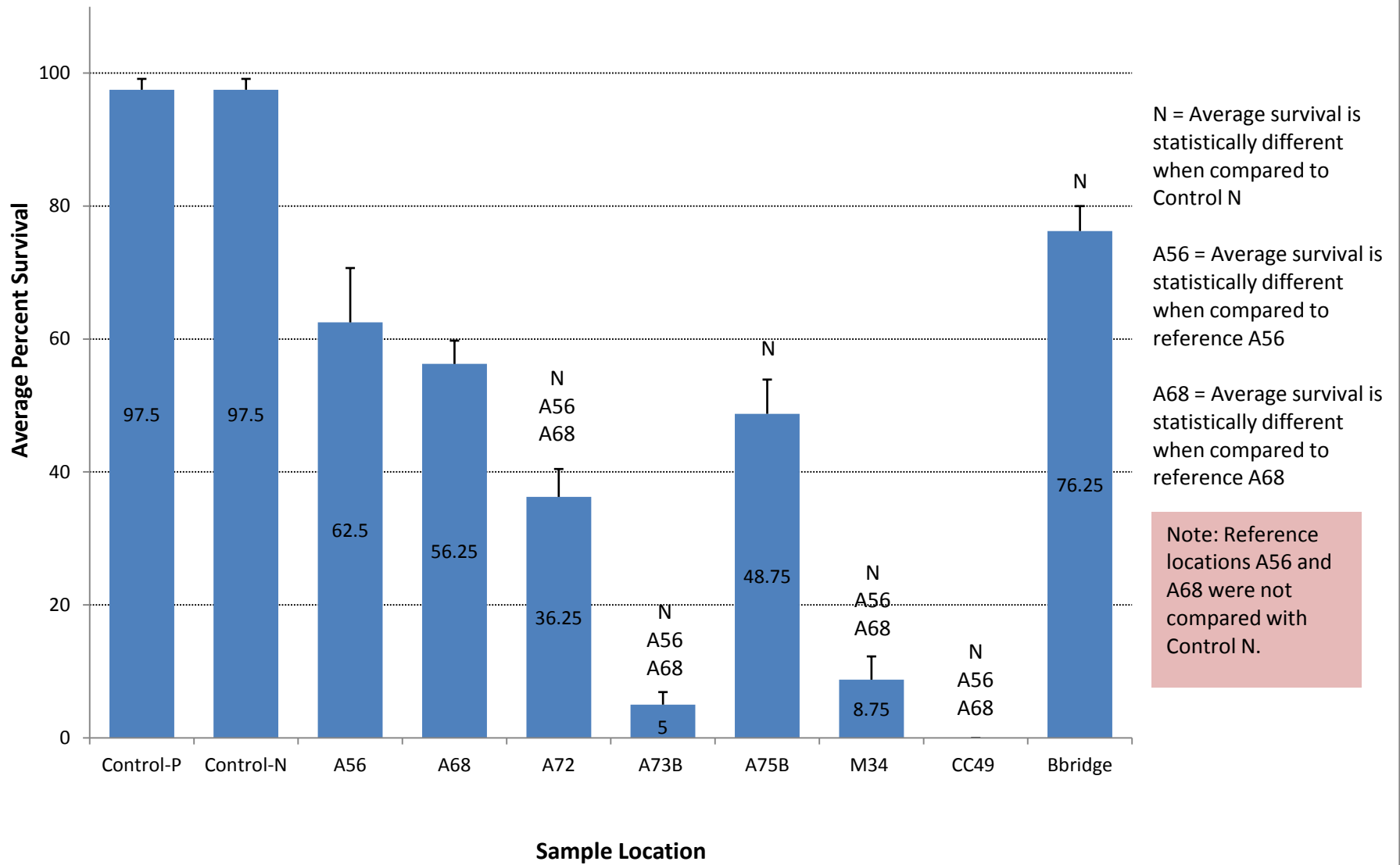
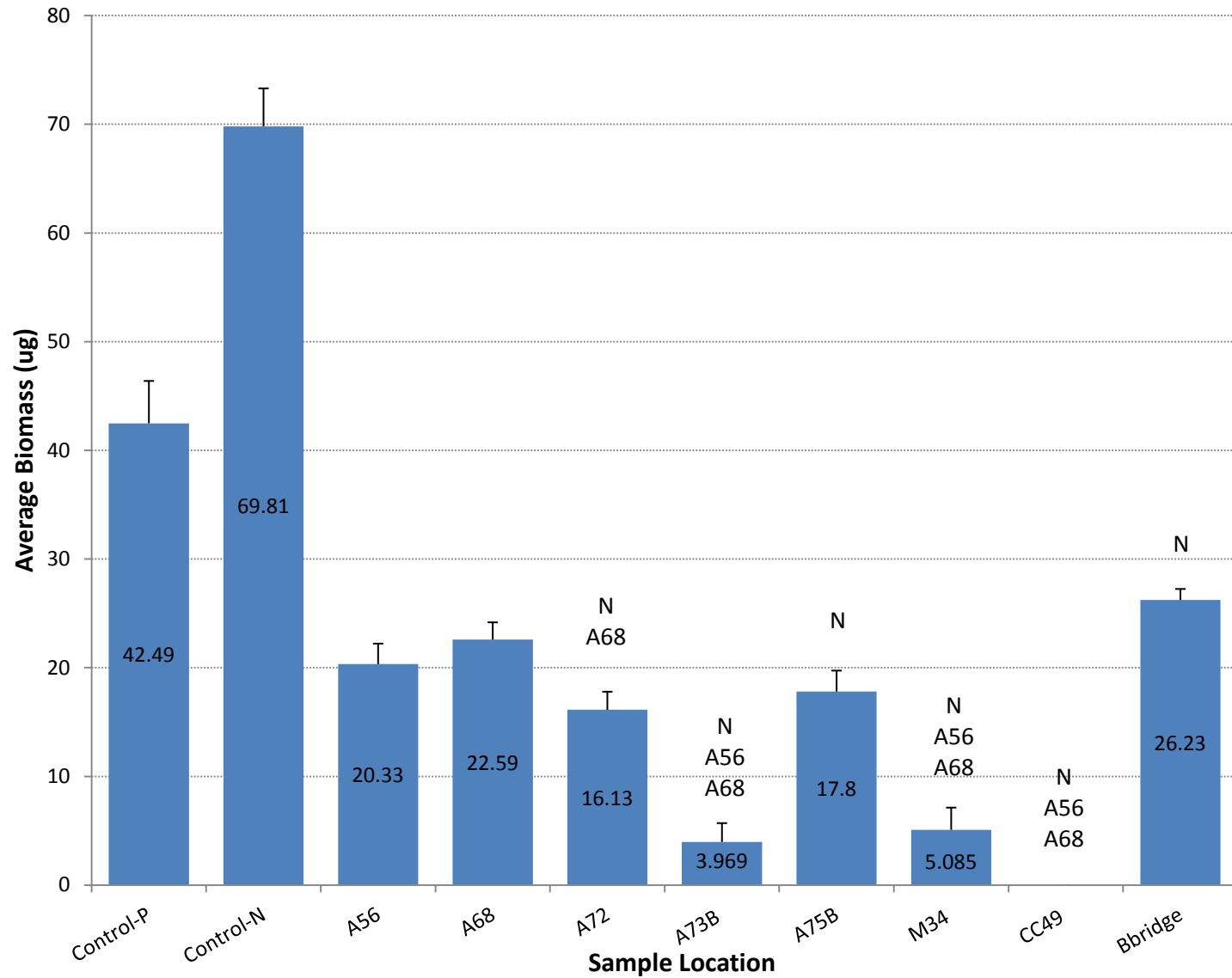


Figure 3.1-2
2012 Upper Animas River Sediment Toxicity Test Using *H. azteca*
Average Biomass + 1 SD per Sampling Location



N = Average biomass is statistically different when compared to Control N

A56 = Average biomass is statistically different when compared to reference A56

A68 = Average biomass is statistically different when compared to reference A68

Note: Reference locations A56 and A68 were not compared with Control N.

Figure 3.2-1
2012 Upper Animas River Concurrent Acute Reference Toxicity Test using *H.azteca* and Zinc Sulfate (ZnSO₄)
Average Percent Survival + 1 SD per Zinc Concentration

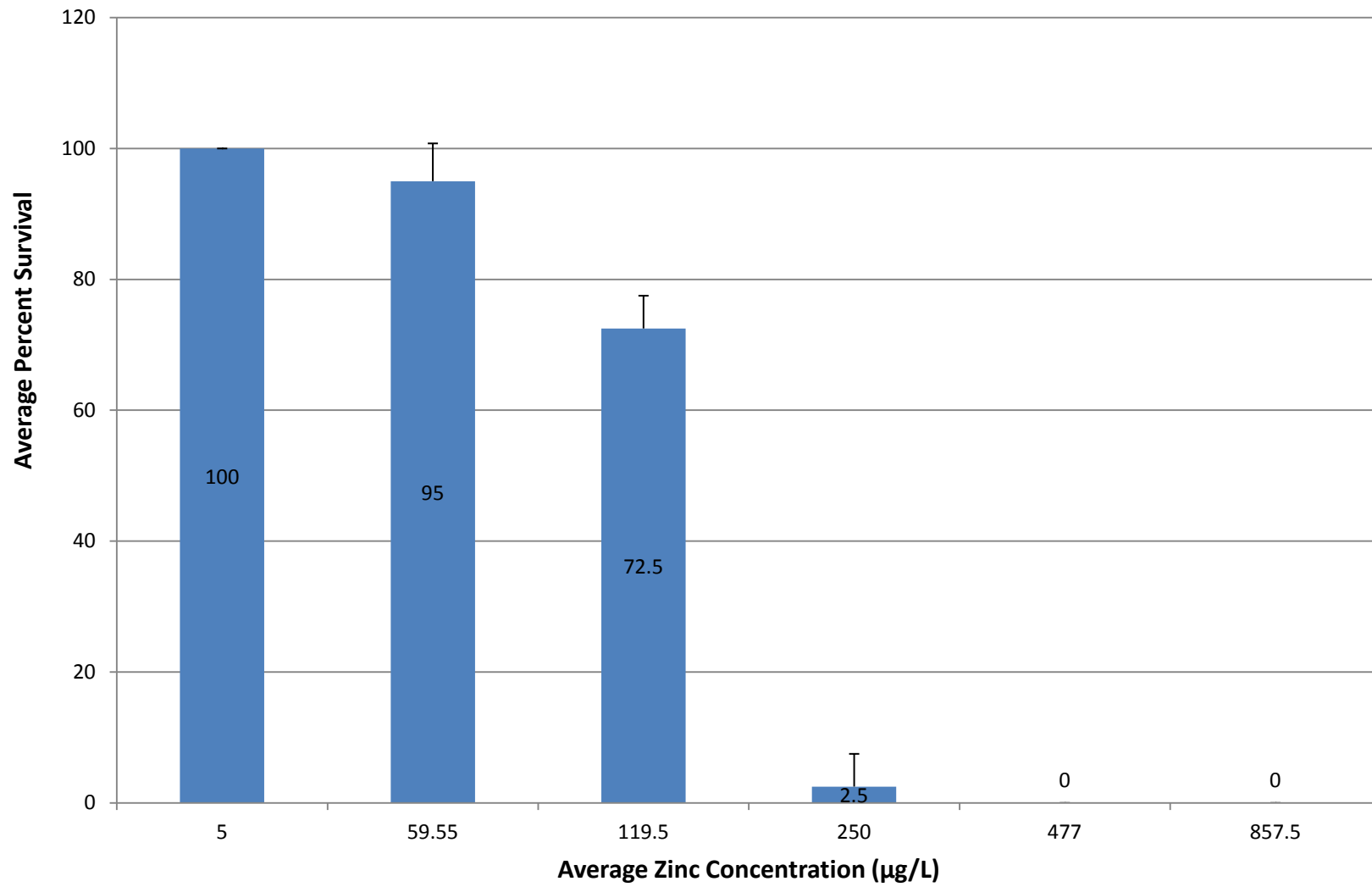
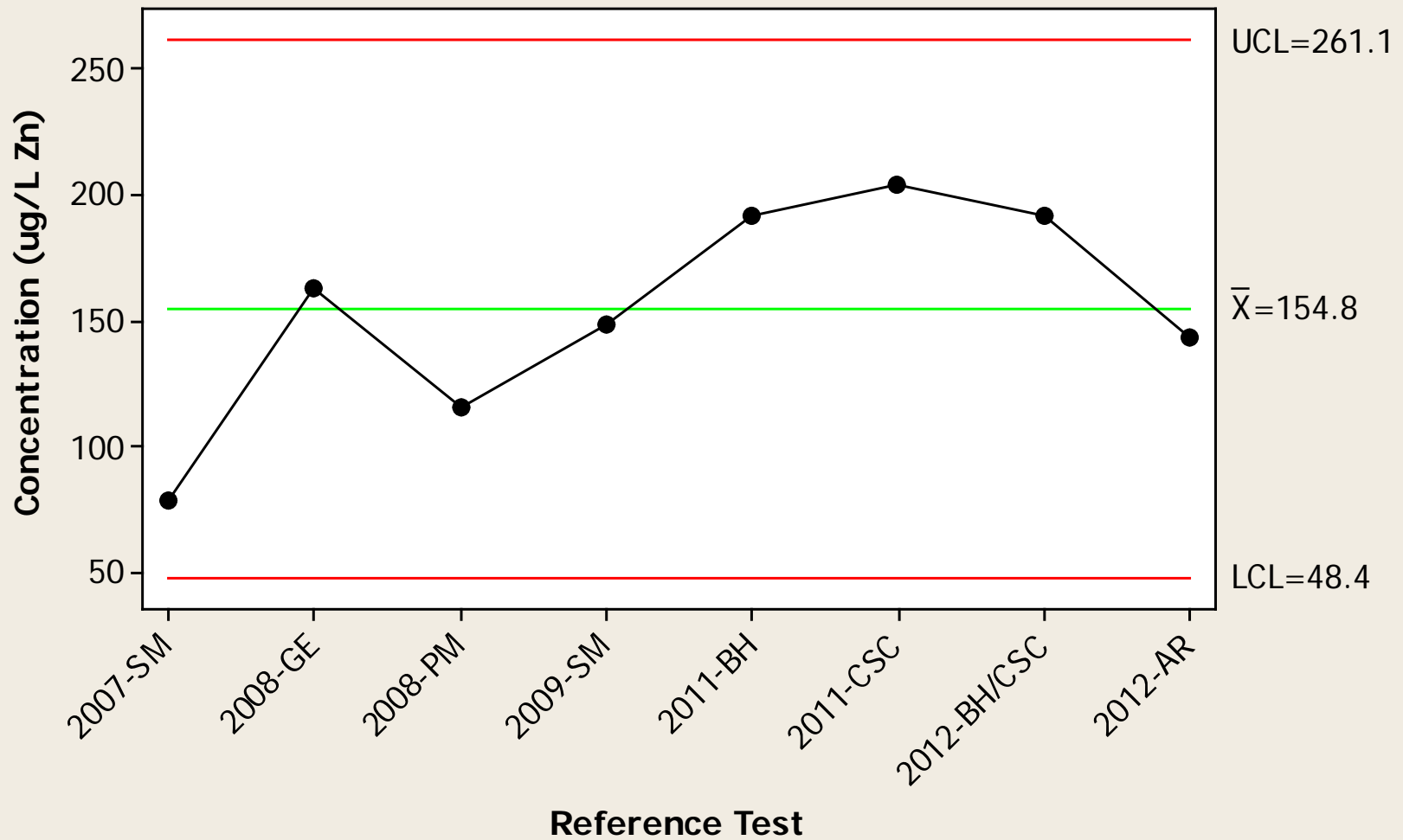


Figure 3.2-2. LC50 Values - Reference Toxicity Tests - H. Azteca



UCL = Upper Control Limit Based off of 3 standard deviations
LCL = Lower Control Limit Based off of 3 standard deviations
X = Mean LC50 values across all tests

Appendices

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control-P-01	pH	7.4									6.66	
Control-P-01	Conductivity (us/cm)	383.4									307.4	
Control-P-01	D.O. (mg/L)	6.98	4.33	4.74	4.64	5.21	5.41	4.58	4.87	5.06	6.39	4.82
Control-P-01	Temp (C)	22.7	22.7	22.4	22.5	22.5	21.9	22.7	22.3	22.7	22.28	22.8
Control-P-01	Hardness	107										87
Control-P-01	Alkalinity	*										*

Control-P-02	pH	7.36									6.69	
Control-P-02	Conductivity (us/cm)	402									312.8	
Control-P-02	D.O. (mg/L)	7.17	4.37	4.65	4.52	5.72	5.25	4.64	4.76	4.98	6.4	4.64
Control-P-02	Temp (C)	22.6	22.7	22.4	22.7	22.7	22.5	22.6	22.3	22.6	22.31	22.8
Control-P-02	Hardness	107										87
Control-P-02	Alkalinity	*										*

Control-P-03	pH	7.46									6.71	
Control-P-03	Conductivity (us/cm)	395.6									270.1	
Control-P-03	D.O. (mg/L)	6.69	4.4	4.87	5.08	5.51	5.2	4.66	4.8	5.48	6.73	4.83
Control-P-03	Temp (C)	22.7	22.7	22.3	22.7	22.3	22	22.6	22.3	22.7	22.21	22.8
Control-P-03	Hardness	107										87
Control-P-03	Alkalinity	*										*

Control-P-04	pH	7.45									6.74	
Control-P-04	Conductivity (us/cm)	309.7									295	
Control-P-04	D.O. (mg/L)	6.88	4.31	4.65	4.93	5.69	5.01	4.83	4.69	5.29	5.28	4.82
Control-P-04	Temp (C)	22.6	22.7	22.4	22.6	22.2	22	22.3	22.2	22.6	22.02	22.8
Control-P-04	Hardness	107										87
Control-P-04	Alkalinity	*										*

Control-P-05	pH	7.44									6.74	
Control-P-05	Conductivity (us/cm)	383.6									322.9	
Control-P-05	D.O. (mg/L)	7.02	4.25	4.85	4.81	5.2	5.24	3.95	4.55	4.96	5.42	4.64
Control-P-05	Temp (C)	22.6	22.9	22.2	22.8	22.5	22	22.8	22.3	22.8	22.36	22.8
Control-P-05	Hardness	107										87
Control-P-05	Alkalinity	*										*

Control-P-06	pH	7.51									6.8	
Control-P-06	Conductivity (us/cm)	295.1									296.4	
Control-P-06	D.O. (mg/L)	7.2	4.41	4.91	5.01	5.42	5.17	4.17	4.32	4.88	5.71	4.59
Control-P-06	Temp (C)	22.6	23	22.5	22.8	22.8	22.2	22.6	22.5	22.8	22.28	22.8
Control-P-06	Hardness	107										87
Control-P-06	Alkalinity	*										*

Control-P-07	pH	7.44									6.77	
Control-P-07	Conductivity (us/cm)	372.9									319.4	
Control-P-07	D.O. (mg/L)	7.03	4.23	4.64	4.77	5.63	5.23	4.37	4.46	4.73	5.42	4.66
Control-P-07	Temp (C)	22.8	23	22.6	22.9	22.8	22.2	22.6	22.5	23	22.4	23
Control-P-07	Hardness	107										87
Control-P-07	Alkalinity											

Control-P-08	pH	7.5									6.82	
Control-P-08	Conductivity (us/cm)	285.6									277.8	
Control-P-08	D.O. (mg/L)	7.08	4.18	4.73	4.49	5.53	5.12	4.43	4.34	4.83	5.19	4.62
Control-P-08	Temp (C)	22	23.1	22.6	22.7	22.8	22.3	22.9	22.6	22.7	22.32	23
Control-P-08	Hardness	107										87
Control-P-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
 End Date 12/20/12
 Water Type MHRW
 Analysts SA, CL, LC

No. of Replicates 8
 Organism *H. azteca*
 No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control-N-01	pH	6.86									5.75	
Control-N-01	Conductivity (us/cm)	1198									402.5	
Control-N-01	D.O. (mg/L)	7.14	4.11	4.73	5.13	5.3	5.27	4.89	4.85	4.97	6.6	5.26
Control-N-01	Temp (C)	22.5	22.8	22.3	22.6	22.5	22.2	22.6	22.5	22.8	22.2	22.8
Control-N-01	Hardness	386										127
Control-N-01	Alkalinity	*										*

Control-N-02	pH	6.92									5.96	
Control-N-02	Conductivity (us/cm)	1246									401.2	
Control-N-02	D.O. (mg/L)	6.6	4.52	4.85	5.15	5.62	5.14	4.83	4.72	5.08	6.22	4.92
Control-N-02	Temp (C)	22.6	22.8	22.3	22.6	22.5	22.1	22.6	22.4	22.8	22.27	22.8
Control-N-02	Hardness	386										127
Control-N-02	Alkalinity	*										*

Control-N-03	pH	6.96									6.13	
Control-N-03	Conductivity (us/cm)	1107									415.3	
Control-N-03	D.O. (mg/L)	6.68	4.63	4.84	5.18	5.22	5.33	4.76	4.7	5.01	6.37	4.99
Control-N-03	Temp (C)	22.1	22.4	21.9	22.3	22.2	21.5	22.4	22.3	22.4	22.09	22.7
Control-N-03	Hardness	386										127
Control-N-03	Alkalinity	*										*

Control-N-04	pH	6.89									6.22	
Control-N-04	Conductivity (us/cm)	1101									384	
Control-N-04	D.O. (mg/L)	6.44	4.49	4.87	5.05	5.3	5.6	4.74	4.57	5.26	6.03	4.88
Control-N-04	Temp (C)	22.2	22.6	22.1	22.3	22.1	22	22.2	22.1	22.6	22.1	22.6
Control-N-04	Hardness	386										127
Control-N-04	Alkalinity	*										*

Control-N-05	pH	6.94									6.36	
Control-N-05	Conductivity (us/cm)	1230									416.1	
Control-N-05	D.O. (mg/L)	6.52	4.39	4.79	5.21	5.74	5.23	4.63	4.28	5.33	6.46	4.94
Control-N-05	Temp (C)	21.9	22.6	21.6	22	22.2	21.3	22.3	22.2	22.4	21.91	22.6
Control-N-05	Hardness	386										127
Control-N-05	Alkalinity	*										*

Control-N-06	pH	7.05									6.39	
Control-N-06	Conductivity (us/cm)	1045									383.1	
Control-N-06	D.O. (mg/L)	5.88	4.5	4.93	5.2	5.71	5.26	4.61	4.53	4.99	6.31	4.81
Control-N-06	Temp (C)	22.07	22.7	21.6	22.2	22.2	21.1	22.3	22	22.6	21.95	22.6
Control-N-06	Hardness	386										127
Control-N-06	Alkalinity	*										*

Control-N-07	pH	7.04									6.49	
Control-N-07	Conductivity (us/cm)	1296									417.1	
Control-N-07	D.O. (mg/L)	6.41	4.41	4.77	5.15	5.71	5.38	4.66	4.72	4.92	6.48	4.91
Control-N-07	Temp (C)	22.3	22.8	22.1	22.1	22.7	21.6	22.5	22.5	23	22.09	22.8
Control-N-07	Hardness	386										127
Control-N-07	Alkalinity	*										*

Control-N-08	pH	7.13									6.53	
Control-N-08	Conductivity (us/cm)	1072									394.4	
Control-N-08	D.O. (mg/L)	6.63	4.38	4.85	5.16	5.78	5.37	4.81	4.78	4.85	6.01	4.81
Control-N-08	Temp (C)	22.2	22.8	22.3	22.5	22.7	21.6	22.5	22.3	22.6	22.12	22.7
Control-N-08	Hardness	386										127
Control-N-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A56-01	pH	7.13									7.06	
A56-01	Conductivity (us/cm)	400.1									318.4	
A56-01	D.O. (mg/L)	4.75	4.95	4.91	5.38	5.4	5.67	4.76	4.69	5.17	6.11	3.93
A56-01	Temp (C)	21.1	22.3	22.3	22.5	22.2	22.2	22.5	22.3	22.6	22.07	23.1
A56-01	Hardness	118										107
A56-01	Alkalinity	*										*

A56-02	pH	7.19									7.07	
A56-02	Conductivity (us/cm)	412									356	
A56-02	D.O. (mg/L)	5.54	4.58	4.94	5.36	5.56	5.43	4.59	4.56	4.91	4.87	4.11
A56-02	Temp (C)	22.1	22.4	22.1	22.2	22.1	21.6	22.2	22	22.3	22.05	22.8
A56-02	Hardness	118										107
A56-02	Alkalinity	*										*

A56-03	pH	7.21									7.1	
A56-03	Conductivity (us/cm)	399.4									359.1	
A56-03	D.O. (mg/L)	2.59	4.75	4.99	5.57	5.66	5.34	4.6	4.54	5.11	5.52	4.08
A56-03	Temp (C)	22.5	22.3	21.8	22	22.2	21.4	22.8	22.4	22.6	22.18	22.8
A56-03	Hardness	118										107
A56-03	Alkalinity	*										*

A56-04	pH	7.22									7.11	
A56-04	Conductivity (us/cm)	417.4									355.1	
A56-04	D.O. (mg/L)	3.26	4.62	4.86	5.63	5.64	5.27	4.62	4.69	5.05	5.15	4.18
A56-04	Temp (C)	22.5	22.4	22.2	22.2	22.2	21.6	22.5	22.1	22.4	22.03	22.8
A56-04	Hardness	118										107
A56-04	Alkalinity	*										*

A56-05	pH	7.28									7.13	
A56-05	Conductivity (us/cm)	386									352.8	
A56-05	D.O. (mg/L)	3.96	4.61	5.12	5.33	5.58	5.3	4.34	4.55	5.14	5.89	3.78
A56-05	Temp (C)	22.5	22	21.8	21.6	21.3	21.2	22.3	21.7	22.4	21.42	22.6
A56-05	Hardness	118										107
A56-05	Alkalinity	*										*

A56-06	pH	7.29									7.14	
A56-06	Conductivity (us/cm)	391.2									336.5	
A56-06	D.O. (mg/L)	5.05	4.63	5.15	5.66	5.85	5.16	4.5	4.68	5.21	4.46	3.98
A56-06	Temp (C)	22.5	22.5	21.9	22	21.8	21.2	22.2	22.1	22.6	21.95	22.7
A56-06	Hardness	118										107
A56-06	Alkalinity	*										*

A56-07	pH	7.3									7.14	
A56-07	Conductivity (us/cm)	389.2									353.1	
A56-07	D.O. (mg/L)	5.19	4.58	5.23	5.54	5.77	5.26	4.45	4.58	5	5.13	3.67
A56-07	Temp (C)	22.5	22.3	21.7	22	21.8	21.8	22.3	22.2	22.6	21.55	22.5
A56-07	Hardness	118										107
A56-07	Alkalinity	*										*

A56-08	pH	7.32									7.15	
A56-08	Conductivity (us/cm)	390.4									331.4	
A56-08	D.O. (mg/L)	6.02	4.62	4.97	5.48	5.95	5.23	4.51	4.63	5.11	4.58	4.48
A56-08	Temp (C)	22.5	22.8	21.8	22	21.8	22.1	22.6	22.1	22.7	21.89	22.5
A56-08	Hardness	118										107
A56-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A68-01	pH	7.43									7.15	
A68-01	Conductivity (us/cm)	404									352.6	
A68-01	D.O. (mg/L)	5.86	3.89	4.49	4.81	5.05	4.49	4.42	4.39	4.4	4.67	4.12
A68-01	Temp (C)	21.1	22	22	21.3	22	21.5	22.7	21.9	22.1	21.72	22.5
A68-01	Hardness	106										103
A68-01	Alkalinity	*										*

A68-02	pH	7.46									7.2	
A68-02	Conductivity (us/cm)	377.3									351.7	
A68-02	D.O. (mg/L)	3.19	3.91	4.83	4.98	5.14	4.9	4.29	4.32	4.91	4.83	4.19
A68-02	Temp (C)	22.1	22	22.1	21.8	22.1	21	22.7	22.1	22.3	21.81	22.7
A68-02	Hardness	106										103
A68-02	Alkalinity	*										*

A68-03	pH	7.49									7.21	
A68-03	Conductivity (us/cm)	381.6									349.4	
A68-03	D.O. (mg/L)	4.18	3.95	4.62	4.8	5.23	5.08	4.32	4.32	4.94	4.21	4.24
A68-03	Temp (C)	21.8	22.4	21.5	21.8	21.7	21.2	22.8	21.3	21.8	21.73	22.7
A68-03	Hardness	106										103
A68-03	Alkalinity	*										*

A68-04	pH	7.52									7.23	
A68-04	Conductivity (us/cm)	367.5									359.1	
A68-04	D.O. (mg/L)	3.73	3.92	4.72	4.98	5.22	4.81	4.12	4.24	4.57	4.55	4.09
A68-04	Temp (C)	21.6	21.8	21	21.5	20.8	21.5	22.5	21.5	22.1	21.49	22.6
A68-04	Hardness	106										103
A68-04	Alkalinity	*										*

A68-05	pH	7.54									7.23	
A68-05	Conductivity (us/cm)	385.5									351.3	
A68-05	D.O. (mg/L)	4.19	3.96	4.68	5.16	5.01	4.97	3.98	4.16	4.73	4.79	4.19
A68-05	Temp (C)	21.8	22.2	21.8	22.1	21.4	21.3	22.7	21.7	22.5	21.95	22.8
A68-05	Hardness	106										103
A68-05	Alkalinity	*										*

A68-06	pH	7.55									7.25	
A68-06	Conductivity (us/cm)	373.3									355	
A68-06	D.O. (mg/L)	3.93	4.2	4.75	5.05	5.45	4.95	3.97	4.1	4.65	5.05	3.96
A68-06	Temp (C)	22	22.3	22	22	21.2	21.8	22.6	22	22.5	21.93	22.8
A68-06	Hardness	106										103
A68-06	Alkalinity	*										*

A68-07	pH	7.55									7.23	
A68-07	Conductivity (us/cm)	391.6									349.2	
A68-07	D.O. (mg/L)	4.4	3.84	4.63	4.88	5.21	4.91	4.13	4.22	4.78	4.6	4.07
A68-07	Temp (C)	22	22.3	22.3	22.2	21.8	21.9	22.7	22.2	22.5	22.17	23.1
A68-07	Hardness	106										103
A68-07	Alkalinity	*										*

A68-08	pH	7.55									7.27	
A68-08	Conductivity (us/cm)	382.1									347.1	
A68-08	D.O. (mg/L)	4.77	4.01	4.49	4.91	5.44	5.09	4.48	4.31	5.27	5.1	4.15
A68-08	Temp (C)	22.3	22.4	22	22.2	21.8	21.8	22.7	22	22.6	22.16	23
A68-08	Hardness	106										103
A68-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A72-01	pH	7.13									7.01	
A72-01	Conductivity (us/cm)	517									377.2	
A72-01	D.O. (mg/L)	5.13	5.27	5.6	5.75	6.1	5.8	4.94	5.11	5.55	5.03	4.86
A72-01	Temp (C)	22	22.5	21.8	22	21.2	21.8	22.6	21.7	22.3	21.84	22.2
A72-01	Hardness	176										113
A72-01	Alkalinity	*										*

A72-02	pH	7.11									7.01	
A72-02	Conductivity (us/cm)	496.3									372.4	
A72-02	D.O. (mg/L)	5.57	5.64	5.78	5.95	6.33	5.64	5.26	5.08	5.51	5.53	4.9
A72-02	Temp (C)	22	22.1	21.3	22.4	21.7	21.5	22.5	21.7	22.3	21.67	22.8
A72-02	Hardness	176										113
A72-02	Alkalinity	*										*

A72-03	pH	7.08									7.01	
A72-03	Conductivity (us/cm)	502.9									375.7	
A72-03	D.O. (mg/L)	5.35	5.52	5.85	6.16	5.66	5.82	5.32	5.17	5.74	6.34	5.02
A72-03	Temp (C)	21.7	21.8	21.3	21.9	21.6	21	22	21.7	22.2	21.53	22.5
A72-03	Hardness	176										113
A72-03	Alkalinity	*										*

A72-04	pH	7.07									7.02	
A72-04	Conductivity (us/cm)	511.4									370.9	
A72-04	D.O. (mg/L)	4.84	5.57	5.74	5.99	5.97	5.66	5.49	5.36	5.67	5.14	5.07
A72-04	Temp (C)	21.3	21.9	21.5	21.2	21.2	21.1	21.8	21.4	22.6	21.59	22.7
A72-04	Hardness	176										113
A72-04	Alkalinity	*										*

A72-05	pH	7.06									7.02	
A72-05	Conductivity (us/cm)	500									375.5	
A72-05	D.O. (mg/L)	5.95	5.4	5.57	5.87	6.17	5.82	5.11	5.15	5.48	6.32	4.83
A72-05	Temp (C)	22.1	22.2	21.7	21.3	21.3	21.5	22.2	22.2	22.7	21.72	22.7
A72-05	Hardness	176										113
A72-05	Alkalinity	*										*

A72-06	pH	7.07									7.01	
A72-06	Conductivity (us/cm)	488.6									370	
A72-06	D.O. (mg/L)	6.02	5.38	5.71	5.85	6.03	5.65	5.04	5.19	5.44	6.22	4.77
A72-06	Temp (C)	21.4	22	22.2	21.3	21.3	21.6	22.2	21.9	22.2	21.71	22.8
A72-06	Hardness	176										113
A72-06	Alkalinity	*										*

A72-07	pH	7.04									7.01	
A72-07	Conductivity (us/cm)	518.9									364.9	
A72-07	D.O. (mg/L)	6.97	5.27	5.62	5.92	5.88	5.75	5.4	5.13	5.54	5.49	4.69
A72-07	Temp (C)	22.5	22.8	22	22.1	22.1	21.7	22.3	22.2	22.4	22.19	22.7
A72-07	Hardness	176										113
A72-07	Alkalinity	*										*

A72-08	pH	7.06									7	
A72-08	Conductivity (us/cm)	491.8									370.3	
A72-08	D.O. (mg/L)	6.75	5.06	5.47	5.86	6.27	5.75	5.27	5.3	5.45	5.8	4.14
A72-08	Temp (C)	22	22.7	22.2	22.3	22.1	21.4	22.4	22.1	22.2	22.11	22.7
A72-08	Hardness	176										113
A72-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*

10-Day Static Renewal Data Sheets

Start Date	12/10/12
End Date	12/20/12
Water Type	MHRW
Analysts	SA, CL, LC

No. of Replicates	8
Organism	<i>H. azteca</i>
No. of Organisms	80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A73B-01	pH	7.48									7.18	
A73B-01	Conductivity (us/cm)	441									358.3	
A73B-01	D.O. (mg/L)	5.19	4.45	5.04	4.97	4.71	5.01	4.38	4.29	4.89	4.19	4.74
A73B-01	Temp (C)	21.6	22.5	22	22.3	21.9	21.7	22.8	22.3	22.5	21.93	22.7
A73B-01	Hardness	120										105
A73B-01	Alkalinity	*										*

A73B-02	pH	7.44									7.19	
A73B-02	Conductivity (us/cm)	388.7									341.4	
A73B-02	D.O. (mg/L)	4.45	4.49	5.18	5.38	5.37	5.16	4.52	4.58	4.46	4.87	4.33
A73B-02	Temp (C)	22	22.5	22	21.9	21.7	21.6	22.5	22	22.2	21.93	22.7
A73B-02	Hardness	120										105
A73B-02	Alkalinity	*										*

A73B-03	pH	7.41									7.17	
A73B-03	Conductivity (us/cm)	429.7									355.6	
A73B-03	D.O. (mg/L)	4.78	4.71	5.13	5.09	5.82	5.46	4.72	4.5	4.15	5.78	4.5
A73B-03	Temp (C)	21.9	22.3	22	22.2	21.6	21.5	22.6	21.6	22.6	21.77	22.7
A73B-03	Hardness	120										105
A73B-03	Alkalinity	*										*

A73B-04	pH	7.37									7.16	
A73B-04	Conductivity (us/cm)	423.4									354.2	
A73B-04	D.O. (mg/L)	4.77	4.7	5	4.98	5.18	5.31	4.76	4.53	4.46	4.4	4.37
A73B-04	Temp (C)	22	22.1	21.8	21.9	21.9	21.5	22.3	21.5	22.3	21.76	22.6
A73B-04	Hardness	120										105
A73B-04	Alkalinity	*										*

A73B-05	pH	7.36									7.14	
A73B-05	Conductivity (us/cm)	435.8									356.1	
A73B-05	D.O. (mg/L)	6.04	4.52	4.85	5.16	5.44	5.06	4.55	4.43	4.84	4.58	4.47
A73B-05	Temp (C)	22	22.3	22.1	22.3	21.7	22	22.6	22.1	22.5	21.99	22.8
A73B-05	Hardness	120										105
A73B-05	Alkalinity	*										*

A73B-06	pH	7.34									7.14	
A73B-06	Conductivity (us/cm)	423.8									353.1	
A73B-06	D.O. (mg/L)	5.31	4.45	5.06	4.96	5.06	5.21	4.63	4.56	5.01	5.03	4.34
A73B-06	Temp (C)	22.1	22.6	21.7	22.3	21.3	21.1	22.7	22.2	22.5	21.84	22.8
A73B-06	Hardness	120										105
A73B-06	Alkalinity	*										*

A73B-07	pH	7.34									7.12	
A73B-07	Conductivity (us/cm)	432.7									356.5	
A73B-07	D.O. (mg/L)	5.64	4.41	5.01	4.9	5.41	5.08	4.67	4.42	4.77	5.66	4.54
A73B-07	Temp (C)	22	22.7	21.9	22.5	21.8	21.9	22.7	22.5	22.8	22.18	23
A73B-07	Hardness	120										105
A73B-07	Alkalinity	*										*

A73B-08	pH	7.32									7.13	
A73B-08	Conductivity (us/cm)	428.2									315.1	
A73B-08	D.O. (mg/L)	5.97	4.36	4.88	4.91	5.53	5.14	4.7	4.53	4.69	5.87	4.48
A73B-08	Temp (C)	22.6	22.5	22.2	22.3	22	21.8	22.6	22.4	22.7	21.92	23
A73B-08	Hardness	120										105
A73B-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*

10-Day Static Renewal Data Sheets

Start Date	12/10/12
End Date	12/20/12
Water Type	MHRW
Analysts	SA, CL, LC

No. of Replicates	8
Organism	<i>H. azteca</i>
No. of Organisms	80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A75B-01	pH	6.95									6.99	
A75B-01	Conductivity (us/cm)	459									362.1	
A75B-01	D.O. (mg/L)	4.46	5.52	5.42	5.86	6.05	5.71	5.13	5.17	5.13	6.52	4.78
A75B-01	Temp (C)	21.8	22.3	21.7	21.8	21.5	21.7	22.2	21.7	22.2	21.53	22.9
A75B-01	Hardness	143										112
A75B-01	Alkalinity	*										*

A75B-02	pH	7									6.98	
A75B-02	Conductivity (us/cm)	475.5									361.5	
A75B-02	D.O. (mg/L)	5.51	5.68	5.61	5.61	6.21	5.74	4.78	4.62	5.36	6.36	4.85
A75B-02	Temp (C)	21.6	22.1	22.2	22.1	21.9	22.2	22.2	22.5	22.7	21.6	23
A75B-02	Hardness	143										112
A75B-02	Alkalinity	*										*

A75B-03	pH	7.02									6.98	
A75B-03	Conductivity (us/cm)	465.6									363.7	
A75B-03	D.O. (mg/L)	4.64	5.72	5.66	5.87	5.85	5.69	5.03	4.69	5.58	6.25	4.82
A75B-03	Temp (C)	21.5	21.4	21	21.6	20.8	21.8	22.1	22	22.3	21.52	22.1
A75B-03	Hardness	143										112
A75B-03	Alkalinity	*										*

A75B-04	pH	7.08									7	
A75B-04	Conductivity (us/cm)	448.8									358.4	
A75B-04	D.O. (mg/L)	5.4	5.34	5.55	5.93	5.86	5.64	5.2	5.37	5.38	5.39	4.76
A75B-04	Temp (C)	21.5	21.6	21.5	21.5	21.5	21.3	22	21.5	22.1	21.45	22.8
A75B-04	Hardness	143										112
A75B-04	Alkalinity	*										*

A75B-05	pH	7.07									6.99	
A75B-05	Conductivity (us/cm)	460.2									361.7	
A75B-05	D.O. (mg/L)	4.97	5.31	5.56	5.62	5.84	5.49	5.06	5.08	5.53	6.16	4.38
A75B-05	Temp (C)	21.9	21.9	21.8	21.2	21.3	21	22.2	21.4	21.8	21.52	22.6
A75B-05	Hardness	143										112
A75B-05	Alkalinity	*										*

A75B-06	pH	7.1									6.99	
A75B-06	Conductivity (us/cm)	468.5									369	
A75B-06	D.O. (mg/L)	5.45	5.24	5.51	5.75	5.94	5.58	4.87	5.26	5.51	6	4.52
A75B-06	Temp (C)	21.7	22.1	21.8	21.2	21.1	21.1	22.2	21.3	22.3	21.77	22.8
A75B-06	Hardness	143										112
A75B-06	Alkalinity	*										*

A75B-07	pH	7.11									7	
A75B-07	Conductivity (us/cm)	460.9									358.7	
A75B-07	D.O. (mg/L)	5.41	5.39	5.27	5.79	5.93	5.48	4.88	5.02	5.42	5.48	4.67
A75B-07	Temp (C)	21.5	22.8	21.8	21.5	21.3	21.4	22.4	21.7	22.4	21.97	22.8
A75B-07	Hardness	143										112
A75B-07	Alkalinity	*										*

A75B-08	pH	7.13									7.02	
A75B-08	Conductivity (us/cm)	461.3									355.6	
A75B-08	D.O. (mg/L)	5.52	5.42	5.53	5.67	5.84	5.62	5.06	5.07	5.58	5.91	4.73
A75B-08	Temp (C)	21.9	22.5	22.2	21.6	21.3	21.5	22.4	21.7	22.4	22.08	22.7
A75B-08	Hardness	143										112
A75B-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
M34-01	pH	6.17									6.22	
M34-01	Conductivity (us/cm)	499.6									368	
M34-01	D.O. (mg/L)	7.27	6.88	6.1	6.45	6.7	6.41	5.9	6.14	5.29	6.82	5.96
M34-01	Temp (C)	21.3	21.2	21.1	21.7	21.2	22.3	22.4	21.2	21.4	21.19	22.6
M34-01	Hardness	158										108
M34-01	Alkalinity	*										*

M34-02	pH	6.21									6.3	
M34-02	Conductivity (us/cm)	502.7									363.9	
M34-02	D.O. (mg/L)	6.67	6.79	6.26	6.51	6.85	6.1	6.03	6.17	6.14	6.83	5.61
M34-02	Temp (C)	21.4	21.2	21	21.5	21.3	21.2	22.8	21.3	21.7	21.42	22.3
M34-02	Hardness	158										108
M34-02	Alkalinity	*										*

M34-03	pH	6.24									6.48	
M34-03	Conductivity (us/cm)	505.2									359.4	
M34-03	D.O. (mg/L)	6.8	6.72	6.16	6.39	6.66	6.34	5.91	6.14	6.45	6.97	5.53
M34-03	Temp (C)	21.4	21.7	21.1	21.3	21.3	21.2	22.5	21.5	21.3	21.27	22.6
M34-03	Hardness	158										108
M34-03	Alkalinity	*										*

M34-04	pH	6.28									6.51	
M34-04	Conductivity (us/cm)	487.2									368.7	
M34-04	D.O. (mg/L)	5.73	6.44	6.3	6.49	6.85	6.16	5.96	6.12	6.24	6.86	5.57
M34-04	Temp (C)	21.5	21.5	21	21.1	21.5	21.5	22.7	21.6	21.3	21.53	22.6
M34-04	Hardness	158										108
M34-04	Alkalinity	*										*

M34-05	pH	6.28									6.55	
M34-05	Conductivity (us/cm)	518.1									368.2	
M34-05	D.O. (mg/L)	6.44	6.52	6.21	6.29	6.69	5.95	5.87	5.91	6.73	6.97	5.27
M34-05	Temp (C)	21.4	21.7	21.5	21.2	21.7	21.5	22.3	21.6	21.8	21.2	22.3
M34-05	Hardness	158										108
M34-05	Alkalinity	*										*

M34-06	pH	6.32									6.56	
M34-06	Conductivity (us/cm)	488.6									366	
M34-06	D.O. (mg/L)	5.57	6.54	6.13	6.38	6.73	6.17	5.56	5.84	6.32	6.77	5.43
M34-06	Temp (C)	21.7	21.5	21.3	21.7	21.4	21.7	22.5	21.6	21.7	21.68	22.3
M34-06	Hardness	158										108
M34-06	Alkalinity	*										*

M34-07	pH	6.32									6.59	
M34-07	Conductivity (us/cm)	506.9									363.3	
M34-07	D.O. (mg/L)	6.38	6.49	6.03	6.31	6.81	6.23	6.01	5.76	6.29	6.65	5.44
M34-07	Temp (C)	21.9	21.6	21.6	21.9	21.5	21.7	22.5	21.9	21.6	21.92	22.6
M34-07	Hardness	158										108
M34-07	Alkalinity	*										*

M34-08	pH	6.35									6.62	
M34-08	Conductivity (us/cm)	481.4									350.5	
M34-08	D.O. (mg/L)	5.54	6.44	5.93	6.27	6.67	6.19	6.04	5.82	6.25	6.48	5.25
M34-08	Temp (C)	21.2	22.3	22.1	22.2	20.9	21.2	22.5	22	21.1	21.94	22.8
M34-08	Hardness	158										108
M34-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
CC49-01	pH	7.27									7.14	
CC49-01	Conductivity (us/cm)	493.6									366.4	
CC49-01	D.O. (mg/L)	6.8	4.49	4.79	5.23	5.56	5.42	5.02	4.85	4.98	4.72	5.92
CC49-01	Temp (C)	22.3	22.3	22	22.2	21.9	21.5	22.5	22.1	22.6	22.08	22.5
CC49-01	Hardness	161										106
CC49-01	Alkalinity	*										

CC49-02	pH	7.2									5.38	
CC49-02	Conductivity (us/cm)	520.3									321.4	
CC49-02	D.O. (mg/L)	5.59	4.44	4.89	4.6	5.5	5.49	5.09	4.84	4.87	6.24	4.84
CC49-02	Temp (C)	21.5	22.6	22.2	22.5	22.2	21.6	22.5	22.2	22.5	22.05	22.7
CC49-02	Hardness	161										106
CC49-02	Alkalinity	*										*

CC49-03	pH	6.65									4.34	
CC49-03	Conductivity (us/cm)	512.1									371.1	
CC49-03	D.O. (mg/L)	6.46	4.62	5.04	4.97	5.67	5.54	5.01	5.02	5.1	4.34	4.83
CC49-03	Temp (C)	21.8	21.9	21.8	21.8	21.5	21.5	22.3	21.8	22.2	21.93	22.6
CC49-03	Hardness	161										106
CC49-03	Alkalinity	*										*

CC49-04	pH	6.42									4.89	
CC49-04	Conductivity (us/cm)	481.5									364.2	
CC49-04	D.O. (mg/L)	6.95	4.53	5.04	4.78	5.68	5.65	5.07	4.81	5.04	5.52	4.41
CC49-04	Temp (C)	22	22.2	22	22.3	21.5	21.3	22.4	21.8	22.2	21.89	22.7
CC49-04	Hardness	161										106
CC49-04	Alkalinity	*										*

CC49-05	pH	6.27									4.19	
CC49-05	Conductivity (us/cm)	499.5									374.8	
CC49-05	D.O. (mg/L)	7.02	4.44	4.8	4.93	5.1	5.52	4.94	5.06	5.12	3.27	4.69
CC49-05	Temp (C)	21.7	22	22	22	21.7	21.2	22.3	21.8	22.3	21.85	22.6
CC49-05	Hardness	161										106
CC49-05	Alkalinity	*										*

CC49-06	pH	6.07									4.79	
CC49-06	Conductivity (us/cm)	503.7									388	
CC49-06	D.O. (mg/L)	7.05	4.58	4.95	5.17	5.34	5.37	4.86	5.07	4.95	5.19	4.47
CC49-06	Temp (C)	21.5	22.3	21.6	22.1	21.8	21.3	22.2	22.1	22.2	21.67	22.5
CC49-06	Hardness	161										106
CC49-06	Alkalinity	*										*

CC49-07	pH	5.93									4.78	
CC49-07	Conductivity (us/cm)	499									366.7	
CC49-07	D.O. (mg/L)	7.11	4.64	4.94	5.23	5.5	5.41	5.04	4.91	5.07	3.63	4.58
CC49-07	Temp (C)	21.8	22.4	21.9	22.3	22.2	21.6	22.5	22.4	22.8	22.09	22.5
CC49-07	Hardness	161										106
CC49-07	Alkalinity	*										*

CC49-08	pH	5.94									5.24	
CC49-08	Conductivity (us/cm)	466									370.2	
CC49-08	D.O. (mg/L)	6.83	4.64	5.05	5.42	5.27	5.3	5.13	5.01	5.03	5.76	4.77
CC49-08	Temp (C)	22.1	22.7	22.3	22.1	22.5	22.2	22.5	22.2	22.7	21.97	22.8
CC49-08	Hardness	161										106
CC49-08	Alkalinity	*										*

Appendix A. December 2012 Animas River Sediment Toxicity Test Using *H. azteca*
10-Day Static Renewal Data Sheets

Start Date 12/10/12
End Date 12/20/12
Water Type MHRW
Analysts SA, CL, LC

No. of Replicates 8
Organism *H. azteca*
No. of Organisms 80

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
BBRIDGE-01	pH	6.52									6.78	
BBRIDGE-01	Conductivity (us/cm)	435.7									359.8	
BBRIDGE-01	D.O. (mg/L)	6.27	5.9	5.8	5.94	6.11	5.64	5.27	5.55	5.87	6.64	4.87
BBRIDGE-01	Temp (C)	21.6	22.2	22.1	22	22.1	22	22.2	22.1	21.2	21.56	22.3
BBRIDGE-01	Hardness	133										106
BBRIDGE-01	Alkalinity	*										*

BBRIDGE-02	pH	6.73									6.93	
BBRIDGE-02	Conductivity (us/cm)	403.1									346.4	
BBRIDGE-02	D.O. (mg/L)	6.12	5.93	5.83	6.18	6.49	5.92	5.22	5.61	5.1	6.56	4.83
BBRIDGE-02	Temp (C)	21.4	22.2	21	21.4	21.7	21.5	22.2	22.1	21	21.74	22.7
BBRIDGE-02	Hardness	133										106
BBRIDGE-02	Alkalinity	*										*

BBRIDGE-03	pH	6.8									6.84	
BBRIDGE-03	Conductivity (us/cm)	429.1									359.6	
BBRIDGE-03	D.O. (mg/L)	6.56	5.89	5.75	6.14	6.61	5.82	5.28	5.25	5.76	6.01	4.97
BBRIDGE-03	Temp (C)	21.7	21.9	21.5	21.5	21.6	21	22.1	21.7	21.8	21.44	22.3
BBRIDGE-03	Hardness	133										106
BBRIDGE-03	Alkalinity	*										*

BBRIDGE-04	pH	6.84									6.86	
BBRIDGE-04	Conductivity (us/cm)	433.9									355.1	
BBRIDGE-04	D.O. (mg/L)	6.76	5.82	5.77	5.94	6.5	6.04	5.45	5.23	5.65	5.33	4.82
BBRIDGE-04	Temp (C)	21.3	22.2	21.6	21.3	21.5	21.6	22.1	22	22.2	21.56	22.7
BBRIDGE-04	Hardness	133										106
BBRIDGE-04	Alkalinity	*										*

BBRIDGE-05	pH	6.9									6.88	
BBRIDGE-05	Conductivity (us/cm)	430.6									354.6	
BBRIDGE-05	D.O. (mg/L)	4.82	5.68	5.5	5.68	6.09	5.83	5.24	5.21	5.71	6.29	4.64
BBRIDGE-05	Temp (C)	21.8	22.2	21.8	21.5	21.8	21.8	22.2	21.5	22.1	21.46	22.6
BBRIDGE-05	Hardness	133										106
BBRIDGE-05	Alkalinity	*										*

BBRIDGE-06	pH	6.96									6.91	
BBRIDGE-06	Conductivity (us/cm)	428.8									361	
BBRIDGE-06	D.O. (mg/L)	5.7	5.67	5.62	5.79	6.23	5.66	4.98	5.13	5.75	5.64	4.71
BBRIDGE-06	Temp (C)	21.7	22.1	21.7	21.5	21.8	21.8	22.3	21.4	22.2	21.71	23
BBRIDGE-06	Hardness	133										106
BBRIDGE-06	Alkalinity	*										*

BBRIDGE-07	pH	6.99									6.95	
BBRIDGE-07	Conductivity (us/cm)	419.9									357.8	
BBRIDGE-07	D.O. (mg/L)	6.37	5.55	5.57	5.62	5.59	5.43	5.28	5.02	5.54	5.96	4.57
BBRIDGE-07	Temp (C)	21.8	22.2	22	22	22	21.8	22.4	22.2	22.5	21.63	22.7
BBRIDGE-07	Hardness	133										106
BBRIDGE-07	Alkalinity	*										*

BBRIDGE-08	pH	7.02									6.96	
BBRIDGE-08	Conductivity (us/cm)	413.6									353.2	
BBRIDGE-08	D.O. (mg/L)	6.53	5.68	5.51	5.55	6.07	5.71	5.21	5.34	5.34	6.03	4.79
BBRIDGE-08	Temp (C)	21.6	22.3	21.3	21.9	22	21.1	22.4	22.2	22.5	21.36	23
BBRIDGE-08	Hardness	133										106
BBRIDGE-08	Alkalinity	*										*

*Alkalinity was not analyzed due to water volume constraints.

Appendix B Reference Toxicity Test

Concurrent with the 2012 Animas River Sediment Toxicity Test

4-Day Static Renewal Data Sheets

Start Date	12/10/12	No. of Replicates	4
End Date	12/14/12	Organism	<i>H. azteca</i>
Water Type	MHRW	No. of Organisms	40
Analysts	SA, CL, LC		

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
Control-01	No. Alive	10	10	10	10	10
Control-01	pH	7.16				7.35
Control-01	Conductivity (us/cm)	320.2				360.7
Control-01	D.O. (mg/L)	7.72	6.1	6.63	5.85	6.85
Control-01	Temp (C)	22.3	22.7	22.3	22.6	22.1
Control-01	Hardness	96				111
Control-01	Alkalinity	*				*

Control-02	No. Alive	10	10	10	10	10
Control-02	pH	7.2				7.38
Control-02	Conductivity (us/cm)	320.3				366.2
Control-02	D.O. (mg/L)	7.66	5.62	6.16	5.63	6.83
Control-02	Temp (C)	22.5	22.8	22.3	22.6	22.3
Control-02	Hardness	96				111
Control-02	Alkalinity	*				*

Control-03	No. Alive	10	10	10	10	10
Control-03	pH	7.25				6.84
Control-03	Conductivity (us/cm)	319.5				361.3
Control-03	D.O. (mg/L)	7.62	5.79	6.49	6.15	6.8
Control-03	Temp (C)	22.4	22.7	22	22.5	22.25
Control-03	Hardness	96				111
Control-03	Alkalinity	*				*

Control-04	No. Alive	10	10	10	10	10
Control-04	pH	7.29				7.06
Control-04	Conductivity (us/cm)	319.5				356.1
Control-04	D.O. (mg/L)	7.61	5.8	6.52	6.14	6.8
Control-04	Temp (C)	22.5	22.7	22	22.5	22.3
Control-04	Hardness	96				111
Control-04	Alkalinity	*				*

Appendix B Reference Toxicity Test

Concurrent with the 2012 Animas River Sediment Toxicity Test

4-Day Static Renewal Data Sheets

Start Date	12/10/12	No. of Replicates	4
End Date	12/14/12	Organism	<i>H. azteca</i>
Water Type	MHRW	No. of Organisms	40
Analysts	SA, CL, LC		

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
6.25%-01	No. Alive	10	10	10	9	9
6.25%-01	pH	7.34				7
6.25%-01	Conductivity (us/cm)	318.8				353.2
6.25%-01	D.O. (mg/L)	7.59	5.96	6.32	5.83	6.81
6.25%-01	Temp (C)	22.4	22.7	21.7	22.2	22.2
6.25%-01	Hardness	97				108
6.25%-01	Alkalinity	*				*

6.25%-02	No. Alive	10	10	10	10	10
6.25%-02	pH	7.36				7.02
6.25%-02	Conductivity (us/cm)	318.9				354.3
6.25%-02	D.O. (mg/L)	7.57	5.74	6.42	5.69	6.82
6.25%-02	Temp (C)	22.45	22.7	21.7	22.2	22.2
6.25%-02	Hardness	97				108
6.25%-02	Alkalinity	*				*

6.25%-03	No. Alive	10	10	10	9	9
6.25%-03	pH	7.39				7.06
6.25%-03	Conductivity (us/cm)	319.6				353.6
6.25%-03	D.O. (mg/L)	7.55	5.84	6.34	6.19	6.83
6.25%-03	Temp (C)	22.3	22.7	21.7	22.2	22.2
6.25%-03	Hardness	97				108
6.25%-03	Alkalinity	*				*

6.25%-04	No. Alive	10	10	10	10	10
6.25%-04	pH	7.43				7.1
6.25%-04	Conductivity (us/cm)	319.7				351.7
6.25%-04	D.O. (mg/L)	7.56	6.04	6.57	5.73	6.83
6.25%-04	Temp (C)	22.5	22.7	21.6	22.3	22.2
6.25%-04	Hardness	97				108
6.25%-04	Alkalinity	*				*

Appendix B Reference Toxicity Test

Concurrent with the 2012 Animas River Sediment Toxicity Test

4-Day Static Renewal Data Sheets

Start Date	12/10/12	No. of Replicates	4
End Date	12/14/12	Organism	<i>H. azteca</i>
Water Type	MHRW	No. of Organisms	40
Analysts	SA, CL, LC		

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
12.5%-01	No. Alive	10	10	10	7	7
12.5%-01	pH	7.45				7.14
12.5%-01	Conductivity (us/cm)	321.1				357.7
12.5%-01	D.O. (mg/L)	7.53	5.71	6.51	5.65	6.76
12.5%-01	Temp (C)	22.5	22.7	22	22.4	22.3
12.5%-01	Hardness	98				108
12.5%-01	Alkalinity	*				*

12.5%-02	No. Alive	10	10	8	7	7
12.5%-02	pH	7.46				7.18
12.5%-02	Conductivity (us/cm)	318.6				352.9
12.5%-02	D.O. (mg/L)	7.58	5.82	6.87	6.24	6.68
12.5%-02	Temp (C)	22.5	22.8	21.8	22.3	22.3
12.5%-02	Hardness	98				108
12.5%-02	Alkalinity	*				*

12.5%-03	No. Alive	10	10	8	8	8
12.5%-03	pH	7.49				7.23
12.5%-03	Conductivity (us/cm)	319.8				350.2
12.5%-03	D.O. (mg/L)	7.59	5.75	6.78	6.3	6.25
12.5%-03	Temp (C)	22.5	22.7	21.6	22.3	22.2
12.5%-03	Hardness	98				108
12.5%-03	Alkalinity	*				*

12.5%-04	No. Alive	10	10	7	7	7
12.5%-04	pH	7.53				7.26
12.5%-04	Conductivity (us/cm)	318.3				355.7
12.5%-04	D.O. (mg/L)	7.59	6.02	6.74	6.2	6.76
12.5%-04	Temp (C)	22.5	22.8	21.6	22.3	22.2
12.5%-04	Hardness	98				108
12.5%-04	Alkalinity	*				*

Appendix B Reference Toxicity Test

Concurrent with the 2012 Animas River Sediment Toxicity Test

4-Day Static Renewal Data Sheets

Start Date	12/10/12	No. of Replicates	4
End Date	12/14/12	Organism	<i>H. azteca</i>
Water Type	MHRW	No. of Organisms	40
Analysts	SA, CL, LC		

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
25%-01	No. Alive	10	3	1	0	-
25%-01	pH	7.53			-	-
25%-01	Conductivity (us/cm)	318.2			-	-
25%-01	D.O. (mg/L)	7.6	5.77	6.43	5.72	-
25%-01	Temp (C)	22.4	22.6	21.5	22.6	-
25%-01	Hardness	97			103	-
25%-01	Alkalinity	*			*	-

25%-02	No. Alive	10	7	4	0	-
25%-02	pH	7.53			-	-
25%-02	Conductivity (us/cm)	318.7			-	-
25%-02	D.O. (mg/L)	7.6	6.12	6.76	5.53	-
25%-02	Temp (C)	22.4	22.7	21.3	22.6	-
25%-02	Hardness	97			103	-
25%-02	Alkalinity	*			*	-

25%-03	No. Alive	10	2	0	0	-
25%-03	pH	7.54			-	-
25%-03	Conductivity (us/cm)	319			-	-
25%-03	D.O. (mg/L)	7.61	5.84	6.6	5.85	-
25%-03	Temp (C)	22.5	22.7	21.2	22.6	-
25%-03	Hardness	97			103	-
25%-03	Alkalinity	*			*	-

25%-04	No. Alive	10	5	1	1	1
25%-04	pH	7.57				7.29
25%-04	Conductivity (us/cm)	318.8				371.3
25%-04	D.O. (mg/L)	7.6	6.1	6.61	5.72	6.77
25%-04	Temp (C)	22.4	22.7	21.2	22.7	22.27
25%-04	Hardness	97				103
25%-04	Alkalinity	*				*

Appendix B Reference Toxicity Test

Concurrent with the 2012 Animas River Sediment Toxicity Test

4-Day Static Renewal Data Sheets

Start Date	12/10/12	No. of Replicates	4
End Date	12/14/12	Organism	<i>H. azteca</i>
Water Type	MHRW	No. of Organisms	40
Analysts	SA, CL, LC		

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
50%-01	No. Alive	10	5	0	-	-
50%-01	pH	7.54			-	-
50%-01	Conductivity (us/cm)	295			-	-
50%-01	D.O. (mg/L)	7.57	6.55	6	-	-
50%-01	Temp (C)	22.5	22.8	22.6	-	-
50%-01	Hardness	97		105		
50%-01	Alkalinity	*		*		

50%-02	No. Alive	10	1	0	-	-
50%-02	pH	7.48			-	-
50%-02	Conductivity (us/cm)	318.5			-	-
50%-02	D.O. (mg/L)	7.61	6.54	6.32	-	-
50%-02	Temp (C)	22.5	22.7	22.6	-	-
50%-02	Hardness	97		105	-	-
50%-02	Alkalinity	*		*	-	-

50%-03	No. Alive	10	1	0	-	-
50%-03	pH	7.61			-	-
50%-03	Conductivity (us/cm)	318.2			-	-
50%-03	D.O. (mg/L)	7.61	6.48	6.2	-	-
50%-03	Temp (C)	22.6	22.7	22.5	-	-
50%-03	Hardness	97		105	-	-
50%-03	Alkalinity	*		*	-	-

50%-04	No. Alive	10	1	0	-	-
50%-04	pH	7.53			-	-
50%-04	Conductivity (us/cm)	318.1			-	-
50%-04	D.O. (mg/L)	7.63	6.31	6.14	-	-
50%-04	Temp (C)	22.6	22.7	22.5	-	-
50%-04	Hardness	97		105	-	-
50%-04	Alkalinity	*		*	-	-

Appendix B Reference Toxicity Test

Concurrent with the 2012 Animas River Sediment Toxicity Test

4-Day Static Renewal Data Sheets

Start Date	12/10/12	No. of Replicates	4
End Date	12/14/12	Organism	<i>H. azteca</i>
Water Type	MHRW	No. of Organisms	40
Analysts	SA, CL, LC		

Replicate ID	Parameter	Day 0	Day 1	Day 2	Day 3	Day 4
100%-01	No. Alive	10	0	-	-	-
100%-01	pH	7.55				-
100%-01	Conductivity (us/cm)	319.1				-
100%-01	D.O. (mg/L)	7.63	6.29	-	-	-
100%-01	Temp (C)	22.5	22.6	-	-	-
100%-01	Hardness	95	98	-	-	-
100%-01	Alkalinity	*	*	-	-	-

100%-02	No. Alive	10	0	-	-	-
100%-02	pH	7.55				-
100%-02	Conductivity (us/cm)	319.1				-
100%-02	D.O. (mg/L)	7.62	6.54	-	-	-
100%-02	Temp (C)	22.4	22.6	-	-	-
100%-02	Hardness	95	98	-	-	-
100%-02	Alkalinity	*	*	-	-	-

100%-03	No. Alive	10	0	-	-	-
100%-03	pH	7.5				-
100%-03	Conductivity (us/cm)	318.8				-
100%-03	D.O. (mg/L)	7.65	6.01	-	-	-
100%-03	Temp (C)	22.4	22.7	-	-	-
100%-03	Hardness	95	98	-	-	-
100%-03	Alkalinity	*	*	-	-	-

100%-04	No. Alive	10	0	-	-	-
100%-04	pH	7.6				-
100%-04	Conductivity (us/cm)	320.7				-
100%-04	D.O. (mg/L)	7.6	6.16	-	-	-
100%-04	Temp (C)	22.4	22.7	-	-	-
100%-04	Hardness	95	98	-	-	-
100%-04	Alkalinity	*	*	-	-	-

*Alkalinity was not analyzed due to water volume constraints.

Attachment 1

CETIS Analytical Reports for the
December 2012 Upper Animas River Sediment Toxicity Test

CETIS Test Data Worksheet

Report Date: 23 Jul-13 12:05 (p 1 of 2)
 Test Code: 15-2164-2344/5AB26B68

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Start Date: 10 Dec-12 Species: Hyalella azteca Sample Code: Control-P
 End Date: 20 Dec-12 Protocol: EPA/600/R-99/064 (2000) Sample Source: Upper Animas River
 Sample Date: 10 Dec-12 Material: Lab Control Sample Station: positive control

Batch Note: Region 8: Upper Animas River H. azteca 10-day sediment toxicity test

Sample Code	Rep	Pos	# Exposed	# Survived	Total Weight-mg	Tare Weight-mg	Pan Count	Mean Length-mm
Control-P	1	74	10	10	207.694	207.2946	10	
Control-P	2	18	10	10	207.8862	207.4937	10	
Control-P	3	33	10	10	207.7784	207.2301	10	
Control-P	4	71	10	9	206.4633	206.1138	9	
Control-P	5	50	10	10	209.611	209.3062	10	
Control-P	6	27	10	10	208.7849	208.2163	10	
Control-P	7	52	10	10	209.2436	208.9422	10	
Control-P	8	76	10	9	206.0131	205.4788	9	
Control N	1	30	10	10	207.723	207.119	10	
Control N	2	34	9	9	207.2429	206.6395	9	
Control N	3	12	11	11	210.1563	209.3316	11	
Control N	4	14	10	10	207.1309	206.2197	10	
Control N	5	2	10	9	208.0925	207.4776	9	
Control N	6	7	10	10	207.7105	207.0491	10	
Control N	7	60	10	9	208.9042	208.251	9	
Control N	8	54	10	10	208.7741	208.054	10	
A56	1	38	10	3	209.7175	209.6037	3	
A56	2	37	10	7	209.4562	209.1891	7	
A56	3	10	10	5	207.9212	207.7473	5	
A56	4	80	10	8	207.0759	206.8522	8	
A56	5	64	10	9	209.2445	209.0023	9	
A56	6	73	10	7	205.6212	205.4031	7	
A56	7	79	10	3	207.9364	207.7913	3	
A56	8	41	10	8	208.7065	208.4639	8	
A68	1	5	10	5	208.234	208.033	5	
A68	2	55	10	6	206.7461	206.5464	6	
A68	3	29	10	7	205.9819	205.69	7	
A68	4	69	10	6	206.9773	206.7561	6	
A68	5	67	10	6	209.5833	209.3364	6	
A68	6	9	10	4	207.4437	207.2956	4	
A68	7	36	10	5	208.7058	208.4766	5	
A68	8	28	10	6	207.923	207.6535	6	
A72	1	1	10	2	207.6846	207.5661	2	
A72	2	39	10	4	207.1808	207.0385	4	
A72	3	72	10	2	206.7494	206.6758	2	
A72	4	75	10	3	206.7789	206.6091	3	
A72	5	51	10	4	207.6777	207.4905	4	
A72	6	58	10	5	205.4616	205.2522	5	
A72	7	13	10	5	205.041	204.86	5	
A72	8	44	10	4	206.038	205.8298	4	
A73B	1	66	10	0	0	208.092	0	
A73B	2	23	10	0	0	205.4375	0	
A73B	3	42	10	0	0	206.4601	0	
A73B	4	25	10	1	205.445	205.3807	1	
A73B	5	68	10	0	0	204.6448	0	
A73B	6	77	10	1	205.8659	205.7678	1	

CETIS Test Data Worksheet

Report Date: 23 Jul-13 12:05 (p 2 of 2)
 Test Code: 15-2164-2344/5AB26B68

Sample Code	Rep	Pos	# Exposed	# Survived	Total Weight-mg	Tare Weight-mg	Pan Count	Mean Length-mm
A73B	7	26	10	1	206.9982	206.8777	1	
A73B	8	59	10	1	206.1551	206.1205	1	
A75B	1	70	10	6	205.9818	205.7897	6	
A75B	2	47	10	6	208.8464	208.6709	6	
A75B	3	8	10	3	208.9266	208.8363	3	
A75B	4	15	10	5	208.7208	208.4917	5	
A75B	5	35	10	5	207.8091	207.6612	5	
A75B	6	49	10	3	206.4732	206.3462	3	
A75B	7	43	10	4	204.0144	203.8098	4	
A75B	8	24	10	7	208.2514	207.994	7	
M34	1	32	10	0	0	209.5943	0	
M34	2	46	10	3	206.5334	206.3873	3	
M34	3	21	10	0	0	206.1762	0	
M34	4	45	10	1	207.6867	207.5722	1	
M34	5	63	10	0	0	208.422	0	
M34	6	17	10	1	206.312	206.3025	1	
M34	7	53	10	1	208.334	208.2543	1	
M34	8	57	10	1	207.9543	207.8973	1	
CC49	1	20	10	0	0	205.0744	0	
CC49	2	40	10	0	0	207.328	0	
CC49	3	16	10	0	0	205.9781	0	
CC49	4	31	10	0	0	205.9107	0	
CC49	5	62	10	0	0	204.6736	0	
CC49	6	22	10	0	0	206.8513	0	
CC49	7	56	10	0	0	204.9534	0	
CC49	8	19	10	0	0	207.0552	0	
Bbridge	1	11	10	9	206.2499	205.9917	9	
Bbridge	2	4	10	8	206.2489	206.0004	8	
Bbridge	3	65	10	8	208.6091	208.2976	8	
Bbridge	4	3	10	8	205.4966	205.253	8	
Bbridge	5	6	10	8	209.2402	208.9439	8	
Bbridge	6	61	10	6	208.8208	208.5834	6	
Bbridge	7	48	10	6	209.1293	208.898	6	
Bbridge	8	78	10	8	208.8923	208.6205	8	

CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 3 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 03-9669-0013	Endpoint: Survival Rate	CETIS Version: CETISv1.8.0
Analyzed: 22 Jul-13 16:25	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 20-0097-5864	Test Type: Survival-Growth	Analyst:
Start Date: 10 Dec-12	Protocol: EPA/600/R-99/064 (2000)	Diluent: Not Applicable
Ending Date: 20 Dec-12	Species: Hyalella azteca	Brine:
Duration: 10d 0h	Source: In-House Culture	Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run					24.9%

Dunnett's Multiple Comparison Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
A56		A72	3.937	2.357	14	0.169	0.0007	Significant Effect
		A73B	9.534	2.357	14	0.169	<0.0001	Significant Effect
		A75B	2.113	2.357	14	0.169	0.0831	Non-Significant Effect
		M34	8.8	2.357	14	0.169	<0.0001	Significant Effect
		CC49	10.67	2.357	14	0.169	<0.0001	Significant Effect
		Bbridge	-2.034	2.357	14	0.169	0.9997	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.18987	1.031645	6	50.17	<0.0001	Significant Effect
Error	1.007528	0.0205618	49			
Total	7.197399	1.052207	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.772	3.195	0.0212	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.967	0.9426	0.1279	Normal Distribution

CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 4 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 03-9669-0013 Endpoint: Survival Rate
 Analyzed: 22 Jul-13 16:25 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
A56	8	0.625	0.537	0.713	0.3	0.9	0.08183	0.2315	37.03%	0.0%
A72	8	0.3625	0.3173	0.4077	0.2	0.5	0.04199	0.1188	32.77%	42.0%
A73B	8	0.05	0.02967	0.07033	0	0.1	0.0189	0.05345	106.9%	92.0%
A75B	8	0.4875	0.4321	0.5429	0.3	0.7	0.05154	0.1458	29.9%	22.0%
M34	8	0.0875	0.0498	0.1252	0	0.3	0.03504	0.0991	113.3%	86.0%
CC49	8	0	0	0	0	0	0	0		100.0%
Bbridge	8	0.7625	0.7222	0.8028	0.6	0.9	0.0375	0.1061	13.91%	-22.0%

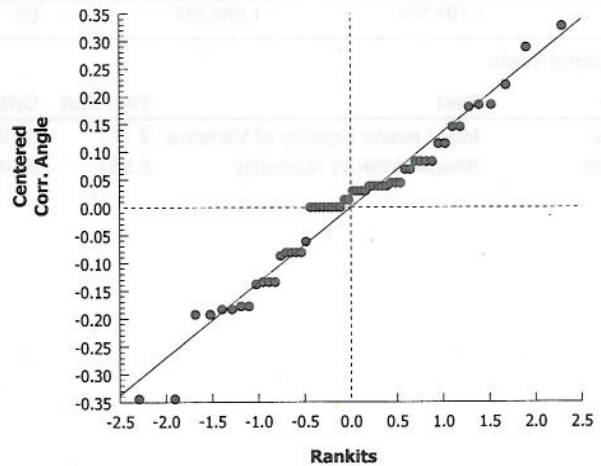
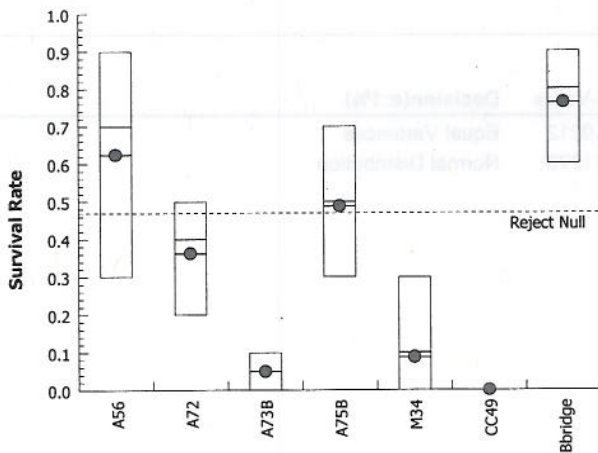
Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
A56	8	0.9238	0.8286	1.019	0.5796	1.249	0.08845	0.2502	27.08%	0.0%
A72	8	0.6415	0.5929	0.6901	0.4636	0.7854	0.04514	0.1277	19.9%	30.56%
A73B	8	0.2403	0.2071	0.2734	0.1588	0.3218	0.0308	0.08711	36.26%	73.99%
A75B	8	0.7723	0.7155	0.8291	0.5796	0.9912	0.05279	0.1493	19.33%	16.4%
M34	8	0.2929	0.2392	0.3466	0.1588	0.5796	0.04991	0.1412	48.2%	68.3%
CC49	8	0.1588	0.1588	0.1588	0.1588	0.1588	0	0	0.0%	82.81%
Bbridge	8	1.07	1.023	1.117	0.8861	1.249	0.04363	0.1234	11.54%	-15.79%

Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
A56	0.3	0.7	0.5	0.8	0.9	0.7	0.3	0.8
A72	0.2	0.4	0.2	0.3	0.4	0.5	0.5	0.4
A73B	0	0	0	0.1	0	0.1	0.1	0.1
A75B	0.6	0.6	0.3	0.5	0.5	0.3	0.4	0.7
M34	0	0.3	0	0.1	0	0.1	0.1	0.1
CC49	0	0	0	0	0	0	0	0
Bbridge	0.9	0.8	0.8	0.8	0.8	0.6	0.6	0.8

Graphics



CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 1 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 13-2360-1137 Endpoint: Survival Rate CETIS Version: CETISv1.8.0
 Analyzed: 22 Jul-13 16:26 Analysis: Parametric-Control vs Treatments Official Results: Yes

Batch ID: 20-0097-5864 Test Type: Survival-Growth Analyst:
 Start Date: 10 Dec-12 Protocol: EPA/600/R-99/064 (2000) Diluent: Not Applicable
 Ending Date: 20 Dec-12 Species: Hyalella azteca Brine:
 Duration: 10d 0h Source: In-House Culture Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run					23.5%

Dunnett's Multiple Comparison Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
A68		A72	3.657	2.357	14	0.1336	0.0017	Significant Effect
		A73B	10.73	2.357	14	0.1336	<0.0001	Significant Effect
		A75B	1.351	2.357	14	0.1336	0.2971	Non-Significant Effect
		M34	9.806	2.357	14	0.1336	<0.0001	Significant Effect
		CC49	12.17	2.357	14	0.1336	<0.0001	Significant Effect
		Bbridge	-3.893	2.357	14	0.1336	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.822945	0.9704908	6	75.46	<0.0001	Significant Effect
Error	0.6301743	0.0128607	49			
Total	6.453119	0.9833515	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.746	3.195	0.1302	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9531	0.9426	0.0294	Normal Distribution

CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 2 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyaella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 13-2360-1137
 Analyzed: 22 Jul-13 16:26
 Endpoint: Survival Rate
 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
A68	8	0.5625	0.5277	0.5973	0.4	0.7	0.03239	0.09161	16.29%	0.0%
A72	8	0.3625	0.3173	0.4077	0.2	0.5	0.04199	0.1188	32.77%	35.56%
A73B	8	0.05	0.02967	0.07033	0	0.1	0.0189	0.05345	106.9%	91.11%
A75B	8	0.4875	0.4321	0.5429	0.3	0.7	0.05154	0.1458	29.9%	13.33%
M34	8	0.0875	0.0498	0.1252	0	0.3	0.03504	0.0991	113.3%	84.44%
CC49	8	0	0	0	0	0	0	0		100.0%
Bbridge	8	0.7625	0.7222	0.8028	0.6	0.9	0.0375	0.1061	13.91%	-35.56%

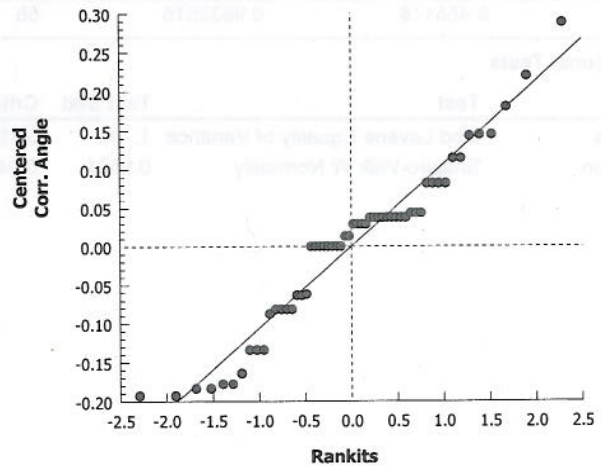
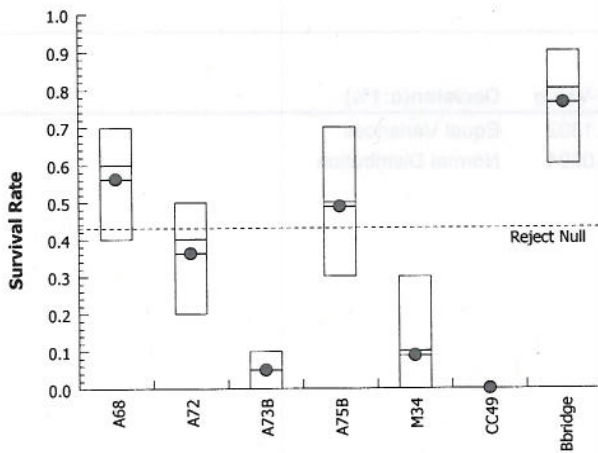
Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
A68	8	0.8489	0.8134	0.8843	0.6847	0.9912	0.03295	0.09319	10.98%	0.0%
A72	8	0.6415	0.5929	0.6901	0.4636	0.7854	0.04514	0.1277	19.9%	24.43%
A73B	8	0.2403	0.2071	0.2734	0.1588	0.3218	0.0308	0.08711	36.26%	71.7%
A75B	8	0.7723	0.7155	0.8291	0.5796	0.9912	0.05279	0.1493	19.33%	9.03%
M34	8	0.2929	0.2392	0.3466	0.1588	0.5796	0.04991	0.1412	48.2%	65.5%
CC49	8	0.1588	0.1588	0.1588	0.1588	0.1588	0	0	0.0%	81.3%
Bbridge	8	1.07	1.023	1.117	0.8861	1.249	0.04363	0.1234	11.54%	-26.0%

Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
A68	0.5	0.6	0.7	0.6	0.6	0.4	0.5	0.6
A72	0.2	0.4	0.2	0.3	0.4	0.5	0.5	0.4
A73B	0	0	0	0.1	0	0.1	0.1	0.1
A75B	0.6	0.6	0.3	0.5	0.5	0.3	0.4	0.7
M34	0	0.3	0	0.1	0	0.1	0.1	0.1
CC49	0	0	0	0	0	0	0	0
Bbridge	0.9	0.8	0.8	0.8	0.8	0.6	0.6	0.8

Graphics



CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 5 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 03-4687-6092 Endpoint: Survival Rate CETIS Version: CETISv1.8.0
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

Batch ID: 20-0097-5864 Test Type: Survival-Growth Analyst:
 Start Date: 10 Dec-12 Protocol: EPA/600/R-99/064 (2000) Diluent: Not Applicable
 Ending Date: 20 Dec-12 Species: Hyalella azteca Brine:
 Duration: 10d 0h Source: In-House Culture Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run					8.26%

Steel Many-One Rank Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Control-P		A72	36	46	14	0	0.0022	Significant Effect
		A73B	36	46	14	0	0.0022	Significant Effect
		A75B	36	46	14	0	0.0022	Significant Effect
		M34	36	46	14	0	0.0022	Significant Effect
		CC49	36	46	14	0	0.0022	Significant Effect
		Bbridge	37	46	14	1	0.0031	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	9.984424	1.664071	6	133.8	<0.0001	Significant Effect
Error	0.6092275	0.01243322	49			
Total	10.59365	1.676504	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.947	3.195	0.0918	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9423	0.9426	0.0098	Non-normal Distribution

Hyaella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 03-4687-6092 Endpoint: Survival Rate
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control-P	8	0.975	0.9574	0.9926	0.9	1	0.01637	0.04629	4.75%	0.0%
A72	8	0.3625	0.3173	0.4077	0.2	0.5	0.04199	0.1188	32.77%	62.82%
A73B	8	0.05	0.02967	0.07033	0	0.1	0.0189	0.05345	106.9%	94.87%
A75B	8	0.4875	0.4321	0.5429	0.3	0.7	0.05154	0.1458	29.9%	50.0%
M34	8	0.0875	0.0498	0.1252	0	0.3	0.03504	0.0991	113.3%	91.03%
CC49	8	0	0	0	0	0	0	0		100.0%
Bbridge	8	0.7625	0.7222	0.8028	0.6	0.9	0.0375	0.1061	13.91%	21.79%

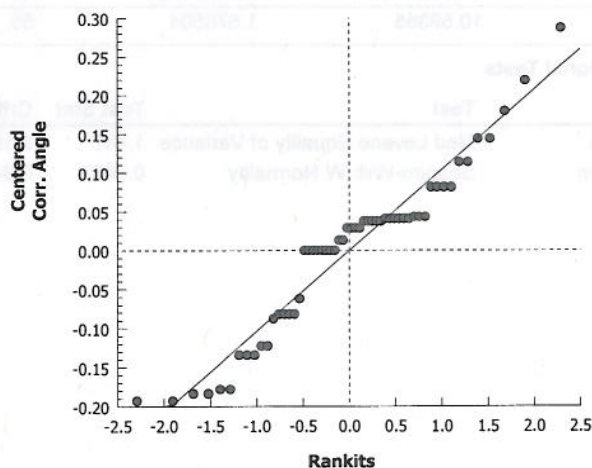
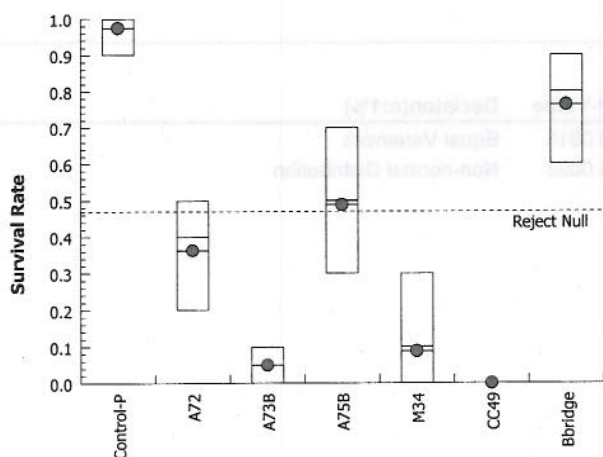
Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control-P	8	1.371	1.343	1.4	1.249	1.412	0.02667	0.07544	5.5%	0.0%
A72	8	0.6415	0.5929	0.6901	0.4636	0.7854	0.04514	0.1277	19.9%	53.22%
A73B	8	0.2403	0.2071	0.2734	0.1588	0.3218	0.0308	0.08711	36.26%	82.48%
A75B	8	0.7723	0.7155	0.8291	0.5796	0.9912	0.05279	0.1493	19.33%	43.68%
M34	8	0.2929	0.2392	0.3466	0.1588	0.5796	0.04991	0.1412	48.2%	78.64%
CC49	8	0.1588	0.1588	0.1588	0.1588	0.1588	0	0	0.0%	88.42%
Bbridge	8	1.07	1.023	1.117	0.8861	1.249	0.04363	0.1234	11.54%	22.0%

Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
Control-P	1	1	1	0.9	1	1	1	0.9
A72	0.2	0.4	0.2	0.3	0.4	0.5	0.5	0.4
A73B	0	0	0	0.1	0	0.1	0.1	0.1
A75B	0.6	0.6	0.3	0.5	0.5	0.3	0.4	0.7
M34	0	0.3	0	0.1	0	0.1	0.1	0.1
CC49	0	0	0	0	0	0	0	0
Bbridge	0.9	0.8	0.8	0.8	0.8	0.6	0.6	0.8

Graphics



CETIS Analytical Report

Report Date: 23 Jul-13 14:20 (p 1 of 4)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 17-8280-6245	Endpoint: Survival Rate	CETIS Version: CETISv1.8.0
Analyzed: 23 Jul-13 14:20	Analysis: Parametric-Control vs Treatments	Official Results: Yes
Batch ID: 20-0097-5864	Test Type: Survival-Growth	Analyst:
Start Date: 10 Dec-12	Protocol: EPA/600/R-99/064 (2000)	Diluent: Not Applicable
Ending Date: 20 Dec-12	Species: Hyalella azteca	Brine:
Duration: 10d 0h	Source: In-House Culture	Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run					8.27%

Dunnett's Multiple Comparison Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Control N		A72	13.09	2.357	14	0.1314	<0.0001	Significant Effect
		A73B	20.28	2.357	14	0.1314	<0.0001	Significant Effect
		A75B	10.74	2.357	14	0.1314	<0.0001	Significant Effect
		M34	19.34	2.357	14	0.1314	<0.0001	Significant Effect
		CC49	21.74	2.357	14	0.1314	<0.0001	Significant Effect
		Bbridge	5.408	2.357	14	0.1314	<0.0001	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	9.982662	1.663777	6	133.8	<0.0001	Significant Effect
Error	0.6092585	0.01243385	49			
Total	10.59192	1.676211	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.929	3.195	0.0948	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9435	0.9426	0.0110	Normal Distribution

CETIS Analytical Report

Report Date: 23 Jul-13 14:20 (p 2 of 4)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 17-8280-6245 Endpoint: Survival Rate
 Analyzed: 23 Jul-13 14:20 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control N	8	0.975	0.9574	0.9926	0.9	1	0.01637	0.04629	4.75%	0.0%
A72	8	0.3625	0.3173	0.4077	0.2	0.5	0.04199	0.1188	32.77%	62.82%
A73B	8	0.05	0.02967	0.07033	0	0.1	0.0189	0.05345	106.9%	94.87%
A75B	8	0.4875	0.4321	0.5429	0.3	0.7	0.05154	0.1458	29.9%	50.0%
M34	8	0.0875	0.0498	0.1252	0	0.3	0.03504	0.0991	113.3%	91.03%
CC49	8	0	0	0	0	0	0	0		100.0%
Bbridge	8	0.7625	0.7222	0.8028	0.6	0.9	0.0375	0.1061	13.91%	21.79%

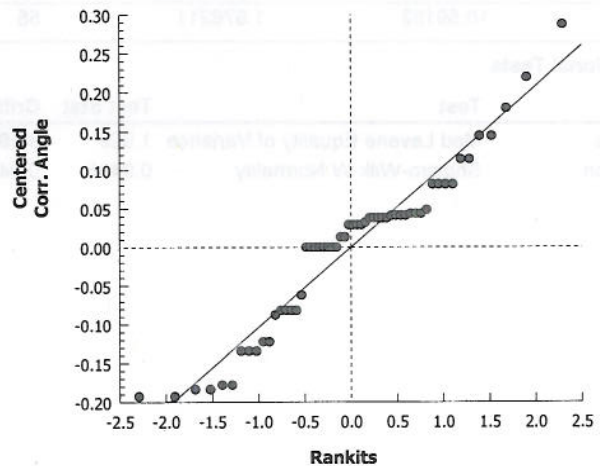
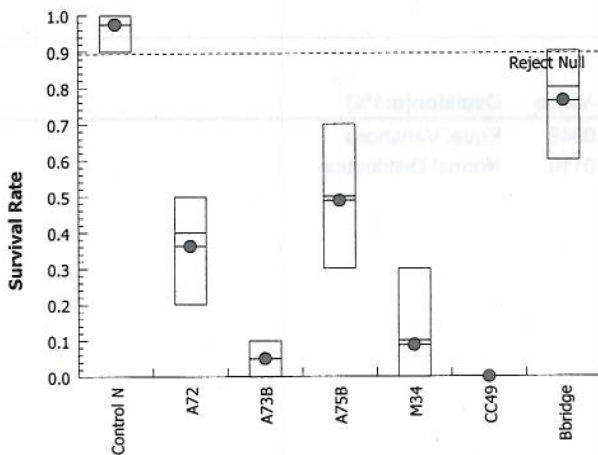
Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control N	8	1.371	1.342	1.4	1.249	1.419	0.02668	0.07547	5.5%	0.0%
A72	8	0.6415	0.5929	0.6901	0.4636	0.7854	0.04514	0.1277	19.9%	53.21%
A73B	8	0.2403	0.2071	0.2734	0.1588	0.3218	0.0308	0.08711	36.26%	82.48%
A75B	8	0.7723	0.7155	0.8291	0.5796	0.9912	0.05279	0.1493	19.33%	43.68%
M34	8	0.2929	0.2392	0.3466	0.1588	0.5796	0.04991	0.1412	48.2%	78.64%
CC49	8	0.1588	0.1588	0.1588	0.1588	0.1588	0	0	0.0%	88.42%
Bbridge	8	1.07	1.023	1.117	0.8861	1.249	0.04363	0.1234	11.54%	21.99%

Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
Control N	1	1	1	1	0.9	1	0.9	1
A72	0.2	0.4	0.2	0.3	0.4	0.5	0.5	0.4
A73B	0	0	0	0.1	0	0.1	0.1	0.1
A75B	0.6	0.6	0.3	0.5	0.5	0.3	0.4	0.7
M34	0	0.3	0	0.1	0	0.1	0.1	0.1
CC49	0	0	0	0	0	0	0	0
Bbridge	0.9	0.8	0.8	0.8	0.8	0.6	0.6	0.8

Graphics



CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 11 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 18-0737-1273	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.0
Analyzed: 22 Jul-13 16:25	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes
Batch ID: 20-0097-5864	Test Type: Survival-Growth	Analyst:
Start Date: 10 Dec-12	Protocol: EPA/600/R-99/064 (2000)	Diluent: Not Applicable
Ending Date: 20 Dec-12	Species: Hyalella azteca	Brine:
Duration: 10d 0h	Source: In-House Culture	Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run					33800.0%

Steel Many-One Rank Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
A56		A72	52	46	14	0	0.1750	Non-Significant Effect
		A73B	37	46	14	0	0.0031	Significant Effect
		A75B	60	46	14	0	0.5231	Non-Significant Effect
		M34	39	46	14	0	0.0062	Significant Effect
		CC49	36	46	14	0	0.0022	Significant Effect
		Bbridge	91	46	14	0	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3025.477	504.2461	6	14.86	<0.0001	Significant Effect
Error	1663.218	33.94323	49			
Total	4688.695	538.1893	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	418	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.7393	0.9426	<0.0001	Non-normal Distribution

Mean Dry Biomass-mg Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
A56	8	0.02033	0.0183	0.02237	0.01138	0.02671	0.001891	0.005349	26.31%	0.0%
A72	8	0.01613	0.01433	0.01792	0.007361	0.02094	0.001665	0.00471	29.21%	20.69%
A73B	8	-10.3	-14.5	-6.111	-20.81	0.01205	3.898	11.02	-107.0%	50780.0%
A75B	8	0.0178	0.01571	0.01988	0.00903	0.02574	0.001938	0.005481	30.79%	12.46%
M34	8	-7.797	-11.9	-3.7	-20.96	0.01461	3.809	10.77	-138.2%	38450.0%
CC49	8	-20.6	-20.64	-20.56	-20.73	-20.47	0.03615	0.1022	-0.5%	101400.0
Bbridge	8	0.02623	0.02514	0.02733	0.02313	0.03115	0.001018	0.00288	10.98%	-29.03%

Hyaella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

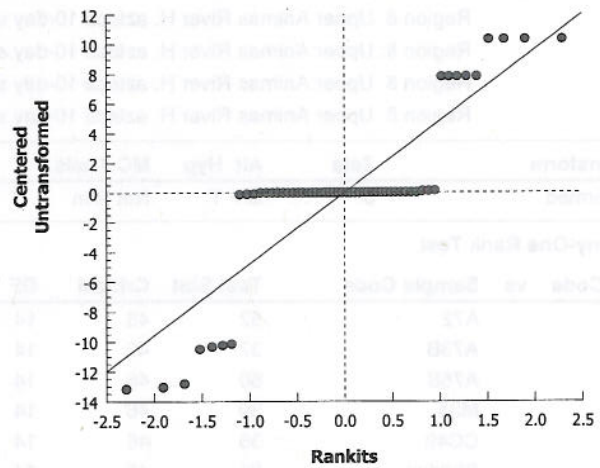
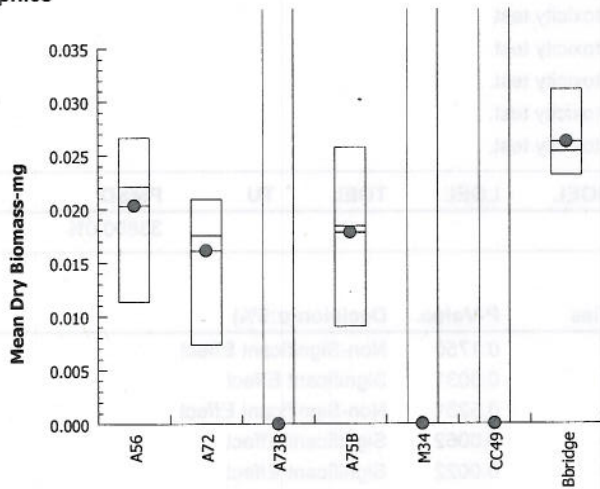
Analysis ID: 18-0737-1273 Endpoint: Mean Dry Biomass-mg
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Mean Dry Biomass-mg Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
A56	0.01138	0.02671	0.01739	0.02237	0.02422	0.02181	0.01451	0.02426
A72	0.01185	0.01423	0.007361	0.01698	0.01872	0.02094	0.0181	0.02082
A73B	-20.81	-20.54	-20.65	0.00643	-20.46	0.00981	0.01205	0.003461
A75B	0.01921	0.01755	0.00903	0.02291	0.01479	0.0127	0.02046	0.02574
M34	-20.96	0.01461	-20.62	0.01145	-20.84	0.0009491	0.00797	0.005701
CC49	-20.51	-20.73	-20.6	-20.59	-20.47	-20.69	-20.5	-20.71
Bbridge	0.02582	0.02485	0.03115	0.02436	0.02963	0.02374	0.02313	0.02718

Graphics



Sample Code	Count	Mean	Std. Dev.	Sum Squares	DF	F Stat	P-Value	Test Stat	Critical	Reject H0	Special Variance	Normal Distribution
A56	8	0.0232	0.0187	0.0257	7	0.0257	0.858	1.031	10.81	< 0.001	0.0001	< 0.001
A72	8	0.0173	0.0152	0.0173	7	0.0173	0.550	1.031	10.81	< 0.001	0.0001	< 0.001
A73B	8	-1.42	0.0105	0.0105	7	-1.42	0.120	1.031	10.81	< 0.001	0.0001	< 0.001
A75B	8	0.0178	0.0188	0.0188	7	0.0178	0.550	1.031	10.81	< 0.001	0.0001	< 0.001
M34	8	-1.87	0.0187	0.0187	7	-1.87	0.120	1.031	10.81	< 0.001	0.0001	< 0.001
CC49	8	-20.6	0.009	0.009	7	-20.6	0.009	1.031	10.81	< 0.001	0.0001	< 0.001
Bbridge	8	0.0258	0.0258	0.0258	7	0.0258	0.858	1.031	10.81	< 0.001	0.0001	< 0.001

CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 9 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 19-8337-4441 Endpoint: Mean Dry Biomass-mg CETIS Version: CETISv1.8.0
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

Batch ID: 20-0097-5864 Test Type: Survival-Growth Analyst:
 Start Date: 10 Dec-12 Protocol: EPA/600/R-99/064 (2000) Diluent: Not Applicable
 Ending Date: 20 Dec-12 Species: Hyalella azteca Brine:
 Duration: 10d 0h Source: In-House Culture Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run					30400.0%

Steel Many-One Rank Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
A68		A72	45	46	14	0	0.0370	Significant Effect
		A73B	36	46	14	0	0.0022	Significant Effect
		A75B	51	46	14	0	0.1452	Non-Significant Effect
		M34	36	46	14	0	0.0022	Significant Effect
		CC49	36	46	14	0	0.0022	Significant Effect
		Bbridge	86	46	14	0	0.9996	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3025.677	504.2795	6	14.86	<0.0001	Significant Effect
Error	1663.218	33.94323	49			
Total	4688.896	538.2228	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	420.4	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.7393	0.9426	<0.0001	Non-normal Distribution

Mean Dry Biomass-mg Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
A68	8	0.02259	0.02089	0.0243	0.01481	0.02919	0.001586	0.004487	19.86%	0.0%
A72	8	0.01613	0.01433	0.01792	0.007361	0.02094	0.001665	0.00471	29.21%	28.63%
A73B	8	-10.3	-14.5	-6.111	-20.81	0.01205	3.898	11.02	-107.0%	45710.0%
A75B	8	0.0178	0.01571	0.01988	0.00903	0.02574	0.001938	0.005481	30.79%	21.22%
M34	8	-7.797	-11.9	-3.7	-20.96	0.01461	3.809	10.77	-138.2%	34610.0%
CC49	8	-20.6	-20.64	-20.56	-20.73	-20.47	0.03615	0.1022	-0.5%	91270.0%
Bbridge	8	0.02623	0.02514	0.02733	0.02313	0.03115	0.001018	0.00288	10.98%	-16.11%

Hyaella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

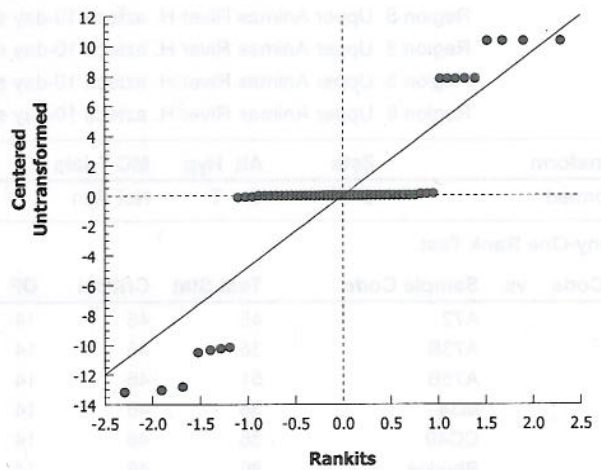
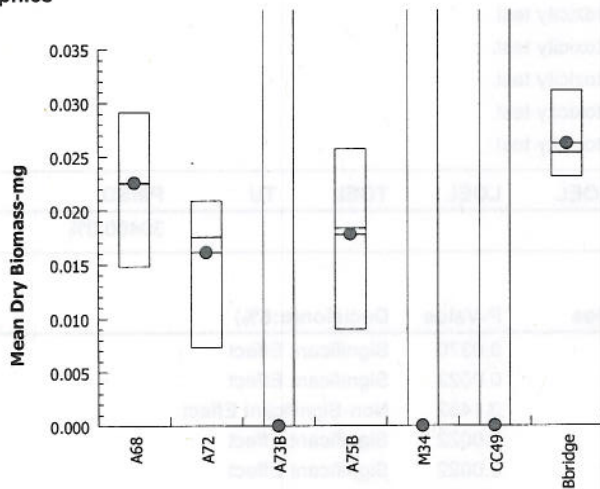
Analysis ID: 19-8337-4441 Endpoint: Mean Dry Biomass-mg
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Mean Dry Biomass-mg Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
A68	0.0201	0.01997	0.02919	0.02212	0.02469	0.01481	0.02292	0.02695
A72	0.01185	0.01423	0.007361	0.01698	0.01872	0.02094	0.0181	0.02082
A73B	-20.81	-20.54	-20.65	0.00643	-20.46	0.00981	0.01205	0.003461
A75B	0.01921	0.01755	0.00903	0.02291	0.01479	0.0127	0.02046	0.02574
M34	-20.96	0.01461	-20.62	0.01145	-20.84	0.0009491	0.00797	0.005701
CC49	-20.51	-20.73	-20.6	-20.59	-20.47	-20.69	-20.5	-20.71
Bbridge	0.02582	0.02485	0.03115	0.02436	0.02963	0.02374	0.02313	0.02718

Graphics



CETIS Analytical Report

Report Date: 22 Jul-13 16:38 (p 7 of 14)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 11-4240-7614 Endpoint: Mean Dry Biomass-mg CETIS Version: CETISv1.8.0
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments Official Results: Yes

Batch ID: 20-0097-5864 Test Type: Survival-Growth Analyst:
 Start Date: 10 Dec-12 Protocol: EPA/600/R-99/064 (2000) Diluent: Not Applicable
 Ending Date: 20 Dec-12 Species: Hyalella azteca Brine:
 Duration: 10d 0h Source: In-House Culture Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run					16200.0%

Steel Many-One Rank Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Control-P		A72	36	46	14	0	0.0022	Significant Effect
		A73B	36	46	14	0	0.0022	Significant Effect
		A75B	36	46	14	0	0.0022	Significant Effect
		M34	36	46	14	0	0.0022	Significant Effect
		CC49	36	46	14	0	0.0022	Significant Effect
		Bbridge	38	46	14	0	0.0044	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3027.443	504.5738	6	14.87	<0.0001	Significant Effect
Error	1663.219	33.94324	49			
Total	4690.662	538.5171	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	408.5	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.7395	0.9426	<0.0001	Non-normal Distribution

Mean Dry Biomass-mg Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control-P	8	0.04249	0.0383	0.04667	0.03014	0.05686	0.003894	0.01101	25.92%	0.0%
A72	8	0.01613	0.01433	0.01792	0.007361	0.02094	0.001665	0.00471	29.21%	62.05%
A73B	8	-10.3	-14.5	-6.111	-20.81	0.01205	3.898	11.02	-107.0%	24350.0%
A75B	8	0.0178	0.01571	0.01988	0.00903	0.02574	0.001938	0.005481	30.79%	58.11%
M34	8	-7.797	-11.9	-3.7	-20.96	0.01461	3.809	10.77	-138.2%	18450.0%
CC49	8	-20.6	-20.64	-20.56	-20.73	-20.47	0.03615	0.1022	-0.5%	48580.0%
Bbridge	8	0.02623	0.02514	0.02733	0.02313	0.03115	0.001018	0.00288	10.98%	38.25%

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

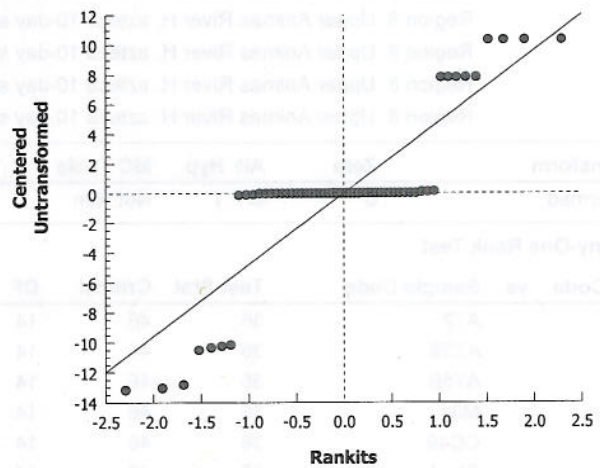
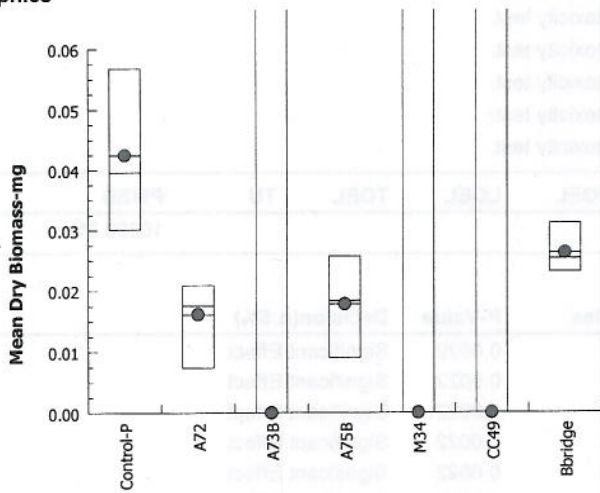
Analysis ID: 11-4240-7614 Endpoint: Mean Dry Biomass-mg
 Analyzed: 22 Jul-13 16:25 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Mean Dry Biomass-mg Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
Control-P	0.03994	0.03925	0.05483	0.03495	0.03048	0.05686	0.03014	0.05343
A72	0.01185	0.01423	0.007361	0.01698	0.01872	0.02094	0.0181	0.02082
A73B	-20.81	-20.54	-20.65	0.00643	-20.46	0.00981	0.01205	0.003461
A75B	0.01921	0.01755	0.00903	0.02291	0.01479	0.0127	0.02046	0.02574
M34	-20.96	0.01461	-20.62	0.01145	-20.84	0.0009491	0.00797	0.005701
CC49	-20.51	-20.73	-20.6	-20.59	-20.47	-20.69	-20.5	-20.71
Bbridge	0.02582	0.02485	0.03115	0.02436	0.02963	0.02374	0.02313	0.02718

Graphics



CETIS Analytical Report

Report Date: 23 Jul-13 14:21 (p 3 of 4)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 11-6603-4398	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.0
Analyzed: 23 Jul-13 14:20	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes
Batch ID: 20-0097-5864	Test Type: Survival-Growth	Analyst:
Start Date: 10 Dec-12	Protocol: EPA/600/R-99/064 (2000)	Diluent: Not Applicable
Ending Date: 20 Dec-12	Species: Hyalella azteca	Brine:
Duration: 10d 0h	Source: In-House Culture	Age:

Sample Code	Sample Comments
Control-P	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Control N	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A56	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A68	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A72	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A73B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
A75B	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
M34	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
CC49	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.
Bbridge	Region 8: Upper Animas River H. azteca 10-day sediment toxicity test.

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	0	C > T	Not Run					9830.0%

Steel Many-One Rank Test

Sample Code	vs	Sample Code	Test Stat	Critical	DF	Ties	P-Value	Decision(α:5%)
Control N		A72	36	46	14	0	0.0022	Significant Effect
		A73B	36	46	14	0	0.0022	Significant Effect
		A75B	36	46	14	0	0.0022	Significant Effect
		M34	36	46	14	0	0.0022	Significant Effect
		CC49	36	46	14	0	0.0022	Significant Effect
		Bbridge	36	46	14	0	0.0022	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3029.877	504.9796	6	14.88	<0.0001	Significant Effect
Error	1663.219	33.94324	49			
Total	4693.096	538.9229	55			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	409.9	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.7394	0.9426	<0.0001	Non-normal Distribution

Mean Dry Biomass-mg Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
Control N	8	0.06981	0.06605	0.07357	0.0604	0.09112	0.003497	0.00989	14.17%	0.0%
A72	8	0.01613	0.01433	0.01792	0.007361	0.02094	0.001665	0.00471	29.21%	76.9%
A73B	8	-10.3	-14.5	-6.111	-20.81	0.01205	3.898	11.02	-107.0%	14860.0%
A75B	8	0.0178	0.01571	0.01988	0.00903	0.02574	0.001938	0.005481	30.79%	74.5%
M34	8	-7.797	-11.9	-3.7	-20.96	0.01461	3.809	10.77	-138.2%	11270.0%
CC49	8	-20.6	-20.64	-20.56	-20.73	-20.47	0.03615	0.1022	-0.5%	29600.0%
Bbridge	8	0.02623	0.02514	0.02733	0.02313	0.03115	0.001018	0.00288	10.98%	62.42%

CETIS Analytical Report

Report Date: 23 Jul-13 14:21 (p 4 of 4)
 Test Code: 5AB26B68 | 15-2164-2344

Hyalella 10-d Survival and Growth Sediment Test

U.S. EPA Region I Lab

Analysis ID: 11-6603-4398
 Analyzed: 23 Jul-13 14:20

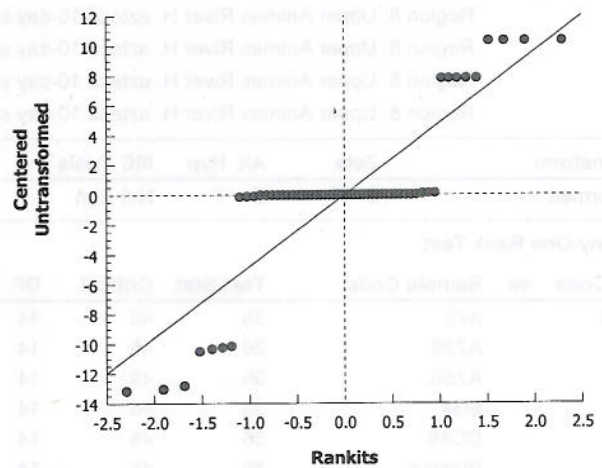
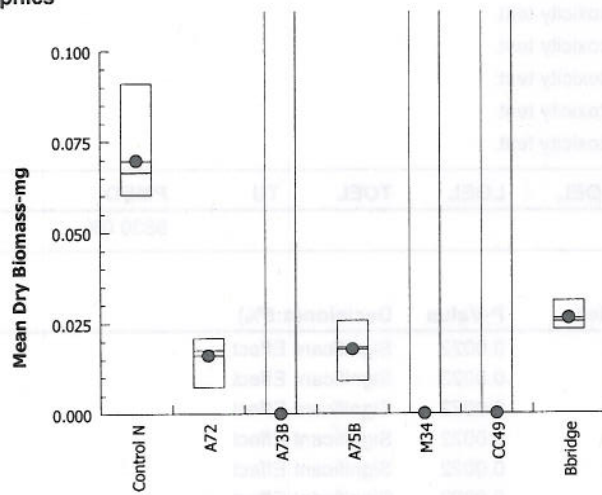
Endpoint: Mean Dry Biomass-mg
 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.0
 Official Results: Yes

Mean Dry Biomass-mg Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
Control N	0.0604	0.06705	0.07497	0.09112	0.06149	0.06614	0.06532	0.07201
A72	0.01185	0.01423	0.007361	0.01698	0.01872	0.02094	0.0181	0.02082
A73B	-20.81	-20.54	-20.65	0.00643	-20.46	0.00981	0.01205	0.003461
A75B	0.01921	0.01755	0.00903	0.02291	0.01479	0.0127	0.02046	0.02574
M34	-20.96	0.01461	-20.62	0.01145	-20.84	0.0009491	0.00797	0.005701
CC49	-20.51	-20.73	-20.6	-20.59	-20.47	-20.69	-20.5	-20.71
Bbridge	0.02582	0.02485	0.03115	0.02436	0.02963	0.02374	0.02313	0.02718

Graphics



Attachment 2

CETIS Analytical Reports for the
Concurrent 96-hr *H. azteca* Reference Toxicity Test to the
December 2012 Upper Animas River Sediment Toxicity Test

CETIS Test Data Worksheet

Report Date: 23 Jul-13 09:33 (p 1 of 1)
 Test Code: 16-6659-1129/63562999

Reference Toxicant 96-h Acute Survival Test

U.S. EPA Region I Lab

Start Date: 10 Dec-12 Species: Hyalella azteca Sample Code: R81210HAARTT
 End Date: Protocol: EPA/600/R-99/064 (2000) Sample Source: Reference Toxicant
 Sample Date: 10 Dec-12 Material: Potassium chloride Sample Station:

Batch Note: Region 8: December 2012 Acute Reference Toxicity Test Using H. azteca (Concurrent with Upper Animas River sediment toxicity test)

Sample Note: Region 8: December 2012 Acute Reference Toxicity Test Using H. azteca (Concurrent with Upper Animas River sediment toxicity test)

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
5	L	1	10	10	10	
5	L	2	21	10	10	
5	L	3	11	10	10	
5	L	4	23	10	10	
59.55		1	2	10	9	
59.55		2	5	10	10	
59.55		3	12	10	9	
59.55		4	18	10	10	
119.5		1	17	10	7	
119.5		2	24	10	7	
119.5		3	3	10	8	
119.5		4	1	10	7	
250		1	16	10	0	
250		2	9	10	0	
250		3	19	10	0	
250		4	13	10	1	
477		1	20	10	0	
477		2	4	10	0	
477		3	14	10	0	
477		4	7	10	0	
857.5		1	8	10	0	
857.5		2	6	10	0	
857.5		3	22	10	0	
857.5		4	15	10	0	

CETIS Analytical Report

Report Date: 23 Jul-13 09:34 (p 1 of 1)
 Test Code: 63562999 | 16-6659-1129

Reference Toxicant 96-h Acute Survival Test

U.S. EPA Region I Lab

Analysis ID: 13-2603-7880 Endpoint: Survival Rate CETIS Version: CETISv1.8.0
 Analyzed: 23 Jul-13 9:34 Analysis: Trimmed Spearman-Kärber Official Results: Yes

Batch ID: 18-0381-5080 Test Type: Survival Analyst:
 Start Date: 10 Dec-12 Protocol: EPA/600/R-99/064 (2000) Diluent: 90 HPW
 Ending Date: Species: Hyalella azteca Brine:
 Duration: N/A Source: In-House Culture Age:

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	2.155	0.02543	143	127.2	160.8

Survival Rate Summary

Calculated Variate(A/B)

Conc-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
5	Lab Water	4	1	1	1	0	0	0.0%	0.0%	40	40
59.55		4	0.95	0.9	1	0.02887	0.05773	6.08%	5.0%	38	40
119.5		4	0.725	0.7	0.8	0.025	0.05	6.9%	27.5%	29	40
250		4	0.025	0	0.1	0.025	0.05	200.0%	97.5%	1	40
477		4	0	0	0	0	0		100.0%	0	40
857.5		4	0	0	0	0	0		100.0%	0	40

Survival Rate Detail

Conc-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
5	Lab Water	1	1	1	1
59.55		0.9	1	0.9	1
119.5		0.7	0.7	0.8	0.7
250		0	0	0	0.1
477		0	0	0	0
857.5		0	0	0	0

Graphics

