FINAL REPORT

Short-Term Toxicity and Developmental Effects of 3-Nitro-1,2,4-Triazol-5-One (NTO) on the Northern Leopard Frog (Lithobates pipiens) in a One-Generation Study

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14. ABSTRACT

The toxicity of 3-nitro-1,2,4-triazol-5-one (NTO), an Insensitive Munitions eXplosive (IMX), to the northern leopard (*Lithobates pipiens*) was assessed in a one-generation study, beginning with newly-hatched larvae and ending with complete or near-complete tail absorption and emergence of the froglet from the water. Because NTO dissolved in water creates acidic conditions (pH ~2.3), the pH of NTO was neutralized with NaOH to a pH of ~7.5 for the definitive study. Test duration was 70 days, during which time larval *L. pipiens* were continuously exposed to water containing NTO at concentrations (time-weighted average) ranging from 190 to 11,350 mg/L. Survival and multiple developmental and growth parameters were measured. All test organisms in the 11,350 mg/L NTO treatment were dead by day 2; those in the 8382 mg/L treatment were dead by the end of the test. The 70-d LC_{50} was 3670 mg/L. The number of organisms with externally-presented front limbs and the number completing metamorphosis to frogs were reduced by NTO; the lowest point estimate was an IC_{10} of 30.04 mg/L for the number completing metamorphosis. Time to front limb eruption and complete metamorphosis were statistically higher in the 3338 mg/L treatment, resulting in a no observed effect concentration (NOEC) of 1346 mg /L, and a lowest observed effect concentration (LOEC) of 3338 mg/L. This impact from NTO may be ecologically significant as delays of even a few days could increase mortality through predation and/or complete loss of the aquatic medium in temporary water bodies. Some pathology was identified in kidneys exposed to 3338 mg/L of NTO. Although examination of a limited number of *L. pipiens* testes suggests possible pathology associated with exposure to NTO, there were insufficient data to make definitive conclusions.

15. SUBJECT TERMS

Lithobates pipiens *Rana pipiens* NTO Aquatic toxicity acute toxicity chronic toxicity explosive

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EXECUTIVE SUMMARY

A study was conducted to determine the effects of 3-nitro-1,2,4-triazol-5-one (NTO) to the northern leopard frog (*Lithobates pipiens*) during a one-generation study. Newly-hatched larvae were exposed to six treatments containing increasing concentrations of NTO (190 – 11,350 mg/L) in a definitive test; a negative control was also included. Prior to initiation of the definitive test, range-finding studies were conducted using both ambient NTO (pH ~3.7 in the highest test treatment) and neutralized (pH-adjusted) NTO with a pH of ~7.5. The range-finding studies were approximately one week long. The definitive study was terminated on day 70 when most of the surviving organisms had developed front limbs and completed metamorphosis into frogs.

The definitive test included six replicates per treatment (with five organisms in each replicate). One replicate chamber from each treatment was terminated on day 14, and a second replicate on day 28, to measure tadpole growth using the metrics snout-to-vent length (SVL) and body width. Exposure in the remaining four replicates continued until the end of the definitive study (70 d). The tests were conducted in an isolated environmental chamber with controlled lighting and temperature. On day 40 of the test larval anurans began presenting front limbs. Organisms with front limbs were transferred from exposure chambers to emergence chambers containing the same concentrations of NTO. Complete metamorphosis (Gosner Stage 46) occurred when the tail was completely or near-completely absorbed. Frogs were examined for internal and external abnormalities; growth was evaluated using a variety of parameters. Histopathological examination was conducted on a limited number of internal organs.

In tests conducted for 9 d with ambient NTO solutions (a pH of approximately 3.7 in the highest test treatment), statistically significant (α=0.05) acute mortality to *L. pipiens* occurred at 383 mg NTO/L in 48 h. The calculated 48-h and 7-d LC_{50}s for *L. pipiens* tadpoles of two different age groups at test initiation (~72 h old and 18 d old) ranged from 236 to 271 mg NTO/L; there was no substantive difference in response between the two age groups.

In pH-adjusted solutions (~7.5 pH), exposure to NTO reduced larval growth over the first two weeks of exposure during the definitive test, resulting in reduced SVL and body width at concentrations as low as ~200 mg/L. By day 28, however, growth of organisms exposed to NTO had caught up with control organisms, with no statistical differences except at 7775 mg/L.

In the 70-d definitive study, all organisms in the highest concentration (time-weighted average [TWA] NTO concentration of 11,350 mg/L) were dead by day 2 of the test. Organisms in the second highest concentration of 8382 mg/L (TWA) showed substantial mortality beginning on day 7 of the test, but some tadpoles survived until day 63 when the last animal in this treatment died. Survival, front limb eruption and number of organisms completing metamorphosis to frogs (Gosner Stage 46) were not significantly ($p>0.05$) affected in concentrations up to 3338 mg/L, which was the highest NTO concentration with surviving organisms at test termination (70 d).

The biomass of four internal organs (liver, heart, kidney and spleen), normalized to total body weight of the individual frogs from which they were extracted, were not significantly reduced at 3338 mg/L or lower. Normalized lung biomass from animals in the 517 mg/L treatment was statistically lower than the control, but lungs in the higher NTO treatments did not demonstrate statistically lower biomass than the control, suggesting the reduction at 517 mg/L was an anomalous statistical response. Kidney biomass from animals exposed to 3338 mg/L was statistically greater than the control, therefore, the lowest selected no observed effect concentration (NOEC) for any of the organ biomass measurements was driven by normalized kidney biomass to be 1346 mg/L.

The mean number of days it took for larval anurans to present front limbs was statistically greater in the 190 mg/L and 3338 mg/L NTO treatments, although not in the 517 and 1346 mg/L treatments. Other temporal metrics, including time to metamorphosis (at Gosner Stage 46), were also statistically greater in the 3338 mg/L treatment, confirming that NTO at a sufficiently high concentration could prolong larval development and delay emergence as adult frogs. The NOEC for the temporal metrics was determined to be 1346 mg/L.

Histopathological examination of liver and kidney samples from the control- and 3338 mg/L NTO-exposed treatments showed no hepatic pathology. There was, however, pathology in renal tissue from the 3338 mg/L NTO treatment, with a higher number of mild to moderate granulomas, relative to control tissue. The small number of animals per group and the presence of lesions in both treated and control animals, however, prevent drawing definitive conclusions regarding the effects of NTO on renal tissues at this time. A limited number of testes samples were also examined. Some sections from the control testes and samples from the 3338 mg/L NTO-exposed group demonstrated fewer, less robust spermatogonia than did other samples. Because of the limited number of samples, no definitive conclusions regarding the effects of NTO exposure can be drawn. Larger control and treatment groups at both Gosner Stage 46 and more mature frog stages may lead to a better understanding of the effects of NTO on the reproductive system in *L. pipiens*.

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QUALITY ASSURANCE STATEMENT

The raw toxicity data and report for the study, *Short-Term Toxicity and Developmental Effects of 3-Nitro-1,2,4-Triazol-5-One (NTO) on the Northern Leopard Frog (Lithobates pipiens) in a One-Generation Study***,** TRE Report No. 15001-468-710, were reviewed for compliance with the test protocol. In addition, the actual conduct of the study was also reviewed for compliance. The results of "in-life" audits were made available to the Study Director and to the laboratory management for corrective action. All other reviews were just reported to the Study Director. The dates of review and of management notification are provided below.

Date Inspected	Type of Inspection	Date Reported to Study Director/ Management
17 June 2015	Phase Audit, including In-Life Study Audit	29 June 2015
9 Oct 2015 – 31 March 2016	QA Review of Laboratory Data and Statistical Analyses	9 Oct 2015 – 31 March 2016
30-31 March 2016	Final Report Evaluation	31 March 2016

Quality Assurance Officer: Anita B. Rehner

Date: 31 March 2016

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The toxicity data and report presented for *Short-Term Toxicity and Developmental Effects of 3-Nitro-1,2,4-Triazol-5-One (NTO) on the Northern Leopard Frog (Lithobates pipiens) in a One-Generation Study***,** TRE Report No. 15001-468-710, comply with the Final Rule for Good Laboratory Practices under the Toxic Substances Control Act (TSCA, 40 CFR Part 792), plus amendments. Characterization, storage stability, and verification of test substance identity are the responsibility of the Study Sponsor and are not encompassed in this compliance statement.

TRE Study Director:

Date: 31 March 2016

1.0 INTRODUCTION

Insensitive Munitions eXplosives (IMX) are designed to be less sensitive to environmental stimuli and less prone to inadvertent detonation during transport and storage. This characteristic is advantageous in that it improves safety by reducing accidental injury not only in active theaters of engagement but also during live-fire training. Diminished IMX sensitivity also reduces risk during manufacture of the compounds. The IMX 3-nitro-1,2,4-triazol-5-one (NTO) was first synthesized in 1905 (Lee and Coburn 1985, as cited in Crouse et al. 2015). In addition to reduced sensitivity, NTO has proven to be nearly equivalent on a performance basis to more traditional explosives (e.g., TNT), making NTO a desirable field munition replacement.

Information on the potential human health and environmental risk characteristics of NTO is sparse, with relevant data being presented in recent reports and professional publications. In general, data on the toxicity of NTO have been generated in short-term studies. Several mammalian studies have been reported. London and Smith (1985, as cited in Crouse et al. 2015) reported that NTO did not induce dermal sensitization in guinea pigs, although a mild skin irritation was induced in rabbits. In the same report, the oral LD_{50} in rats and mice was reported to be <5 g/kg. An acute inhalation study of NTO aerosols (0.18 mg/L) indicated no mortality, clinical signs of toxicity or gross pathological changes in rats (Crouse and O'Neill 2013, as cited in Crouse et al. 2015). Crouse et al. (2015) conducted 14-d and 90-d studies of the effects of NTO on rats when the material was introduced by gavage. They reported that body mass and food consumption decreased in male rats at the 2000 mg/kg-d dose. In the 90-d study, while there were no effects to food consumption, body mass or neurobehavioral metrics up to 1000 mg/kg-d, testes mass was significantly reduced at doses of 315 and 1000 mg/kg-d.

Haley et al. (2009) evaluated the toxicity of NTO on *Ceriodaphnia dubia* (freshwater cladoceran) and *Selenastrum capricornutum* (a green alga, now known as *Pseudokirchneriella subcapitata*). They reported 48-h LC_{50}s of 62 and 460 mg NTO/L when *C. dubia* were exposed to ambient (pH not adjusted) and pH-adjusted NTO solutions, respectively. In a 7-d short-term chronic study, the reported IC_{20} and IC_{50} were 51 and 57 mg NTO/L, respectively, with a 7-d no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) of 34 and 66 mg NTO/L, respectively. The green alga proved less sensitive to NTO. The NOEC and LOEC for *S. capricornutum* were 1375 mg/L and 2680 mg/L of NTO, respectively, and the 96-h median inhibition concentration (IC_{50}) was 3465 mg/L of NTO.

Reddy et al. (2011) assessed the genotoxicity of NTO through a series of tests including the Ames test, Chinese Hamster Ovary (CHO) cell chromosome aberration test, mouse lymphoma mutagenesis test and rat micronucleus test. They reported that NTO was not mutagenic, nor did

it induce chromosomal aberrations in CHO cells. They also reported no statistically significant induction of micronucleated reticulocytes relative to the control.

Amphibians are ubiquitous in wetlands that are often found on or near military facilities where energetics may be used. Amphibians have been recognized as particularly vulnerable to certain chemical stressors in the environment due to unique physiological and behavioural characteristics, such as relatively thin integument and significant dependence on dermal gas exchange (Johnson and Salice 2009). Stanley et al. (2015) evaluated the toxicity of traditional explosives 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) as well as IMX compounds NTO and 2,4-dinitroanisole (DNAN) using the northern leopard frog, *Lithobates* (=*Rana*) *pipiens*. They found that NTO was relatively less toxic than the traditional energetic compounds as well as DNAN. A survival LOEC for NTO obtained in a 28-d chronic bioassay was reported as 5 mg/L.

In the current study, larval (≤ 24 h) *L. pipiens* were exposed in water to a series of NTO concentrations ranging from approximately 200 mg/L to >11,000 mg/L. In addition to shorter-term range-finding tests (up to nine days in duration), the definitive study was continued for 70 d plus extra days to allow some tadpoles to complete metamorphosis into frogs. Nineteen developmental and growth measurements were evaluated at the end of the 70-d exposure as well as during the test to assess in-life effects as the tadpoles grew. Organisms were also dissected for internal examination and assessment of impacts to individual organs, including biomass and limited pathology.

2.0 METHODS

2.1 Preparation of the Initial Stock Solution

Crystalline NTO (99.48% purity, Lot # BAE11L375-061) was obtained from BAE Systems and received at the Army Public Health Center (PHC). An initial super stock solution of NTO was prepared at PHC by suspending approximately 1,400 g of NTO in 4 gallons of PHC in-house deionized water in a 5-gallon high-density polyethylene (HDPE) carboy. The super stock solution suspension (~1.4 x 10^6 mg/15.2 L) was saturated, resulting in much of the NTO settling to the bottom of the carboy. This method of initial preparation was selected as an appropriate manner of supplying and safely transporting the amount of NTO needed for the test. The NTO stock solution for testing (without particulate NTO) was obtained by siphoning liquid from the carboy after it had sat, undisturbed, for a minimum of one hour. Additional information on obtaining the NTO test solution, pH adjustment and creation of additional NTO stock is included in Section 2.4.

2.2 Preparation of Dilution Water

Ultrapure, deionized water was used to prepare test dilution water. The primary laboratory water source for the laboratory is the City of Fort Collins public potable water supply. The municipal tap water was dechlorinated by passage through carbon filtration and two mixed bed ion exchange cartridges (U.S. Filter, Snellville, GA, USA) prior to treatment through a Milli-Q (EMD-Millipore, Billerica, MA, USA) system, which is used for the production of high purity water. The Milli-Q system consists of a fixed bed activated carbon cartridge, ion exchange cartridges, an organic scavenger cartridge, and a 0.45 µm final filter. Final deionized water has a resistivity of ≥ 18 MΩ.

The reconstituted Moderately Hard Water (MHW) (USEPA 2002) used for dilution and control water was prepared by adding reagent-grade salts to deionized water. The added salts were $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$ and KCl (Sigma-Aldrich, St. Louis, MO, USA; Alfa Aesar, Ward Hill, MA, USA). Final hardness and alkalinity ranges of the batches of MHW used as dilution water were 86-90 mg/L as $CaCO_3$ and 57–61 mg/L as $CaCO_3$, respectively.

2.3 Test Organisms

The test species was *Lithobates pipiens* Schreber (1782). Still often referred to as *Rana pipiens*, the northern leopard frog was separated from the *Rana* genus and placed into the genus *Lithobates* (Frost et al. 2006; Crother 2012). Eggs of *Lithobates pipiens* were obtained from Carolina Biological (Burlington, NC, USA). The fertilized eggs were shipped in sealed plastic bags containing oxygenated water. Upon receipt the eggs were examined for initial appearance and the unopened bags were placed in a water bath at 20 ± 2°C. After approximately 48 h, viable embryos were showing visible elongation. Approximately 72 h after receipt (~96 h after fertilization) embryos were released from the bag into an aquarium containing approximately 1 L of MHW that had been brought to 20 ± 2°C. The amount of MHW was gradually increased over 24 h until it made up ≥ 75% of the total liquid in the aquarium. Hatching of healthy larvae was complete at approximately 120-140 h post-fertilization at Gosner Stage 20, at which time the external larval gills are clearly visible and blood circulation is apparent through the gill filaments (Gosner 1960).

2.4 Preparing NTO Solutions

NTO was received as a saturated super stock solution in a 5-gal, high-density polyethylene (HDPE) carboy. Undissolved NTO lay on the bottom of the carboy. The NTO solution was acidic, with a pH of ~2.3. NTO solution for testing was siphoned from the original carboy into 1-gallon clean, low-density polyethylene (LDPE) jars. The siphon (a 5 mL glass pipet attached to a flexible tube) was always held above the bottom of the original carboy to prevent siphoning of solid NTO particles. A 9-d acute toxicity test with dilutions of unadjusted NTO (pH ~2.3) was conducted with *L. pipiens*; all other studies were conducted with dilutions of pH-adjusted NTO.

To raise the pH of the NTO solution, a portion of the siphoned liquid (usually 2.5 to 3 L) was transferred to a 4 L glass beaker. The beaker was placed on a stir plate and a magnetic stir bar was added to the beaker. While mixing at moderate speed (a setting of 5 or 6 on the stir plate), sodium hydroxide (NaOH) was added. Concentrated (10 N) NaOH was usually added although more dilute (e.g., 1 N or 0.1 N) NaOH was added when pH was approaching the target value of 7.5 ± 0.3 pH units). The NTO solution was yellow, changing from a lighter yellow to a darker yellow following pH adjustment (Photographs 1a-1c).

When NTO solution in the original container was siphoned to within approximately 4 cm of the bottom, more water was added. Approximately 4 gal of MHW were added to the original container each time new NTO solution was prepared. A large (~3 to 5 cm) stir bar was added to the original container and the mixture was stirred overnight (minimum of 10 h) at a moderate speed. The next morning the stir plate was turned off and the solution was allowed to settle for several hours before the newly mixed solution was siphoned out as described above.

2.5 Analysis of NTO Concentrations

Samples for analysis of NTO concentration were collected from each test treatment on day 0 (test initiation), test termination (day 70 or earlier if all organisms died or were transferred to emergence chambers) and at each renewal of test solutions (Wednesday of each week through test day 49). Composites of new (freshly prepared aliquots of each concentration) and old (test solutions from the previous renewal day) solutions were collected. Individual replicate samples of old solutions were also collected from select treatments on certain days to evaluate inter-replicate variability. Samples were withdrawn by pipette, placed in labeled, 10-mL borosilicate vials and stored in the dark at 4°C. The following composite samples were quantified for NTO concentration: day 0 (new), day 28 (new and old), day 49 (new and old) and day 70 (old). Analytical samples from selected replicates were collected on day 28 and analyzed individually, as were replicate control samples collected at test termination

Determination of NTO concentrations was completed using High Performance Liquid Chromatography (HPLC) at the U.S. Army Engineer Research and Development Center (ERDC) in Vicksburg, MS. EPA Method 8330 was used, modified for NTO as described in Russell et al. (2014). Analysis of each set of samples included a laboratory control sample (LCS), matrix spike and matrix spike duplicate. The method detection limit in most cases was 0.005 mg NTO/L, and the reporting limit was 0.02 mg NTO/L.

2.6 Range-Finding Tests

Range-finding studies were conducted prior to the definitive test to determine the appropriate concentration range of NTO that would elicit a response from the test organisms. Several range-finding tests were conducted with dilutions of pH-adjusted NTO and ambient NTO (no pH adjustment). Two of the ambient tests and two of the pH-adjusted tests are described here; two age-groups were tested with both ambient NTO and pH-adjusted NTO. One group of organisms was 18 d post-hatch at test initiation while the other was ~72 h post-hatch at test initiation.

2.6.1 Ambient pH Range-Finding Test

Five NTO concentrations plus a MHW control were tested, with two replicates of five organisms each in each treatment. The test system was the same for each of the tests, except for the age of the test organisms. The test concentrations in each of the two tests, as a percent of the saturated NTO stock solution, were 0 (control), 0.31, 0.635, 1.25, 2.5 and 5%, which were equivalent to 0, 24.5, 47.9, 95.8, 191.5 and 383 mg NTO/L. NTO concentrations in the highest and lowest treatments were measured; the intermediate treatments are calculated based on the

high concentration (383 mg/L). The tests were conducted for nine days; the endpoint was survival. The tests were conducted static, without renewal.

2.6.2 pH-adjusted Range-Finding Test

The pH of the NTO was adjusted (as described in Section 2.4) to approximately 7.5 prior to test initiation. Three NTO concentrations plus a MHW control were tested, with two replicates of five organisms each in each treatment. The test system was the same for each of the tests, except for the age of the test organisms. The test concentrations in each of the two tests, as a percent of the saturated NTO stock solution, were 0 (control), 12.5, 25 and 50%, which were equivalent to 0, 943, 1840 and 3710 mg NTO/L. NTO concentrations were measured in all NTO treatments. The tests were conducted for nine days; the endpoint was survival. The tests were conducted static, without renewal.

2.7 Definitive Tests

The definitive test was initiated when the age of the larval *L. pipiens* was ≤ 24 h post-hatch. The initial test chambers were 500 mL plastic (PETE, polyethylene terephthalate) cups containing 250 mL of test solution. Test concentrations were, as a percent of the saturated NTO stock solution, 0 (control), 1.28, 3.2, 8.0, 20, 50 and 75%, which were equivalent to NTO concentrations of approximately 0, 200, 500, 1300, 3050, 8000 and 11,500 mg/L. To prepare the test solutions, an appropriate volume of pH-adjusted NTO stock solution was mixed with MHW in an LDPE cubitainer where it was manually shaken for a minimum of 10 seconds before being added to each cup.

At test initiation there were six replicates, each containing five organisms. Four of those replicates (A – D) were randomized and placed on a foam board into which appropriate-sized holes had been pre-drilled to hold cups in place. Replicates E and F were placed in a separate board but were not randomized. Replicate E (one for each concentration) and replicate F (one for each concentration) were in-life sacrificial test replicates to be terminated on days 14 and 28, respectively, to measure larval growth. Other than placement and early termination, replicates E and F were treated the same as the other replicates.

After addition of the test solution to each chamber, organisms were added by gently pipetting them from the holding aquarium using a glass pipette and bulb. They were released into the test chamber just under the surface of the water. A new pipette was used for each treatment. Tadpoles were at Gosner Stage 21 to 22 at test initiation, with left and right external gills still clearly visible.

The test was conducted in a walk-in environmental chamber with independent (from the rest of the laboratory) lighting and environmental controls. Test boards were placed on shelves, each

with its own light source (Photograph 2). All chambers were covered with plexiglass sheets. Fluorescent lighting was provided at a mean light intensity of 100 to 200 foot-candles at the water's surface, with a 16 h light:8 h dark photoperiod.

On day 7 of the test, organisms were transferred to larger (2.5 gal) glass aquaria containing 3 L of test solution. The 500 mL cups were used for the first week of the test in order to reduce the amount of initial liquid needed and thus preserve the available NTO. For replicates A-D, the 2.5-gal aquaria, each labeled with the test number, NTO treatment and replicate, were randomly placed on shelves within the environmental chamber (Photograph 3). Replicates E and F were placed on separate shelves but were not randomized.

All test chambers were covered with plexiglass sheets. Small holes drilled into the plexiglass covers allowed insertion of a 1 mL pipette for aeration, which was initiated on day 2 of the test when feeding began (see Section 2.7.2). The 1 mL pipettes were connected to air pumps with clear, plastic tubing. Aeration pipettes were positioned so the tip was 1 to 3 cm under the water's surface. Flow rate was adjusted to maintain an even stream of bubbles without excessive agitation of the water.

2.7.1 Water Quality Monitoring

On the day of test initiation (day 0) pH, dissolved oxygen (DO) and temperature were measured in replicates A, B, C and D. These same parameters plus conductivity were also measured in a composite sample of all six replicates (A-F). Hardness and alkalinity (both as mg/L of $CaCO_3$) were measured on day 0 in the control and high NTO treatment. However, the yellow color of the NTO solution interfered with measurement of hardness since the endpoint for that method is color change from red to blue.

Because of the length of the test and the frequency of renewal (every seven days through day 49), multiple batches of MHW were used. Hardness, alkalinity, total ammonia (mg/L as N) and total residual chlorine (mg/L) were measured in each batch of MHW (Table 2-1).

Water quality monitoring was conducted on Monday, Wednesday and Friday of each week. Temperature, DO, pH and conductivity were measured in a composite sample (water from each active replicate) from each treatment. Temperature was measured using a calibrated, digital stick thermometer and pH was measured using a Oakton PCSTestr Model 35. Dissolved oxygen was measured using a YSI Model 51B and specific conductance was determined using a ThermoScientific Orion Star A112. The pH, DO and conductivity meters were calibrated each day before use.

On the day of test solution renewal (Wednesday), temperature, pH, DO and conductivity were measured in each of the freshly-prepared “new” solutions and in the “old” solutions (test water in the exposure chambers from the previous renewal).

2.7.2 Feeding

Feeding began when the tadpoles reached Gosner Stage 25, that is, when there were no longer any visible lateral gill openings; this occurred on test day 2. Organisms were fed Tetrafin® Goldfish Flakes (Tetra GMBN, Melle, Germany) which had been ground to a fine consistency using a mortar and pestle. Preliminary feeding studies with *L. pipiens* yielded information on the appropriate amount of food to give the tadpoles. Too much food resulted in cloudy water and low DO, but too little hindered organism growth, as evidenced by the size of the tadpole and hind limb growth. A set amount (by weight) of food was added to each test chamber by using a set of stainless steel measuring spoons, each containing a different amount of ground Tetrafin in a level scoop. The approximate mass of food delivered by each spoon was calculated as the mean of three replicate dry weight measurements. The amount of provided food increased during the study according to a pre-established schedule (Table 2-2). Food was not provided on the day of test termination. When added, food in the spoon was sprinkled on top of the water over a small area. Tadpoles of *L. pipiens* were observed feeding from the surface, occasionally with bodies completely inverted, as well as from the bottom of the aquarium.

2.7.3 Test Solution Renewal

Test solutions were renewed every seven days through day 49 on Wednesday. On the day of test solution renewal, pH-adjusted NTO stock solution was mixed with MHW at the relative amounts to produce the target test concentrations. Freshly-mixed test solutions were placed in 5-gal LDPE cubitainers and then placed in the environmental chamber to allow them to come to test temperature. Approximately 80% of the liquid in each test chamber was removed during each renewal by siphoning through a glass pipet and flexible tubing. Excess food and waste material was also removed during siphoning. New solution was added after siphoning by gently pouring it down the side of the test chamber to minimize disturbance. Food was added to each chamber after renewal was completed. After day 49, test chambers were not renewed because most of the solid NTO material had been dissolved in the day 49 renewal water and therefore subsequent batches might have significantly lower NTO concentrations following mixing.

2.7.4 Biological Monitoring, Front Limb Eruption, Frog Emergence

Test chambers were examined daily, although number of surviving organisms was only recorded Monday through Friday. On day 6 of the test (Tuesday, May 26, 2015) biological observations were not made. Dead organisms were removed daily. Beginning on day 40 of the

test (Monday, June 29, 2015) tadpoles began to display erupted front limbs, beginning with two organisms in the control treatment and one in the 517 mg/L NTO treatment. On day 45 and all remaining test days following, the total number of surviving organisms, number that were transferred to emergence chambers and number remaining in the exposure chambers were recorded daily, including on the weekend. The presence of front limbs on a new tadpole (not previously observed) was noted on the data sheets.

Development of the tadpoles was categorized using the system proposed by Gosner (1960). In the Gosner classification system, anuran developmental stages range from 1 (fertilization) to 46 (metamorphosis to froglet essentially complete). Some important interim events during development include embryo elongation (stages 15-16), hatching and full development of external gill filaments (stage 20), disappearance of external gills (stage 25), hind limb bud appearance (stage 26), completion of toe development (stage 38) and front limb eruption (stage 42).

Tadpoles that developed front limbs were transferred to emergence chambers from the original exposure chamber two to five days after the original observation of the presence of front limbs. Emergence chambers were pre-cleaned, rectangular polypropylene containers (2,250 mL) into which some (approximately 500 mL) of the respective exposure solution had been transferred (each emergence chamber corresponded to an exposure chamber). The emergence chambers were set at an angle on a wood 2x4 so the elevated end was above the liquid level (Photograph 4). As late-stage tadpoles completed metamorphosis they could crawl out of the water onto the dry surface (Photograph 5). Test organisms remained in the emergence chambers until they completed metamorphosis (tail absorption at Gosner Stage 46) or died prior to completion of metamorphosis.

2.7.5 Test Termination

2.7.5.1 Replicates E and F (14- and 28-Day Tests)

On day 14, the replicate E chambers were terminated; on day 28 the replicate F chambers were terminated. Live tadpoles were removed with a net and placed in MHW containing tricaine methanesulfonate (“MS-222”) (Western Chemical, Inc., Ferndale, WA, USA). Approximately 10 to 15 mL of a 2 g/L MS-222 solution were added to 100 mL of MHW. After movement of the animals had stopped, they were taken out of the MS-222 solution and rinsed with deionized water. Snout-to-vent length (SVL) and body width were measured on each animal to the nearest 0.5 mm. SVL is the distance from the anterior tip of the snout to the vent or anal opening, which is located on the ventral side of the tail, close to the body. Width is the widest part of the tadpole body.

2.7.5.2 Replicates A-D

Upon visual determination that an individual organism had completed metamorphosis (tail was completely or near-completely absorbed), it was removed from the emergence chamber with a net and placed in MHW containing MS-222. Approximately 5 to 10 mL of a 2 g/L MS-222 solution were added to 50 mL of MHW. After movement of the animal had stopped, it was removed, rinsed with deionized water, blotted gently with a paper towel and weighed to the nearest 0.1 mg on an AND Model FR-300 MKII analytical balance. Euthanized organisms were then placed in 10% osmotically-balanced formalin for later examination.

Except for organisms that had already been transferred to emergence chambers, the test was terminated on day 70. All tadpoles that were still in the exposure chambers (and did not have front limbs) were euthanized in MS-222 and preserved in 10% osmotically-balanced formalin. Organisms in emergence chambers on day 70 remained there until they completed metamorphosis (and then were treated as described earlier) or died prior to completing metamorphosis.

2.7.5.3 Growth Measurements and Internal Examination

Snout-to-vent length (SVL) and the lengths of limb segments were measured on preserved animals with a vernier caliper to the nearest 0.01 mm (Photograph 6). Lengths of the left and right radio-ulna, left and right femur, and left and right tibio-fibula were determined. As these measurements were taken on intact, undissected animals, the recorded lengths are only estimates of the actual bone length, but measurement methods were consistent among specimens. The radio-ulna was measured as the distance from the outside of the elbow to the wrist (proximal end of the pad of the front foot); the femur was measured as the distance from the approximate head of the femur at the pelvis mid-line (from a ventral aspect) to the knee joint; the tibio-fibula was measured from the knee joint to the tarsal/tibio-fibula joint.

All frogs from replicates A-D were dissected for internal examination. Any visible sign of internal lesions or abnormalities was noted. Liver, heart, spleen, lungs and kidneys were excised from each animal in replicates A-C, rinsed with deionized water, blotted with a paper towel or Kimwipe®, and weighed to the nearest 0.01 mg on a Sartorius BP 210D analytical balance. After weighing, organs were placed in small vials containing 5-10% formalin. Testes were excised from identifiable male frogs in replicates A-D. Because the gonads of the frogs were very small, they were sometimes lost when excising other organs, especially the kidneys to which both ovaries and testes tended to be attached. When testes were identified, they were removed and placed in small vials containing 5-10% formalin. Because of their very small size, testes could not be accurately weighed using the available balance(s).

2.8 Histopathology

Kidneys and livers from five control animals and five animals from the 3338 mg/L NTO treatment (both organs taken from the same animals) were sent to the Division of Toxicologic Pathology, Army Public Health Center (Provisional) for histologic processing and evaluation. Samples were preserved in 10% formalin. Samples were processed according to the standard operating procedures of the laboratory, which incuded being embedded in paraffin, sectioning onto microscope slides and staining for microscopic examination.

Testes were removed from four male animals from the control treatment and four from the 3338 mg/L treatment (not necessarily the same animals from which other organs were taken) and sent to the Division of Toxicologic Pathology, Army Public Health Center (Provisional) for histologic processing and evaluation. Testes samples from two control and three 3338 mg/L frogs were successfully processed onto slides for microscopic examination.

2.9 Data Analysis

Proportional data (survival, number achieving front limb eruption, number achieving metamorphosis, organ biomass) were transformed using arc-sine square root prior to analysis. One-way analysis of variance (ANOVA) was used to determine the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) for each of the measured metrics, at a significance level (α) of 0.05. Data were evaluated using two-tailed tests and/or one-tailed tests with alternative null hypotheses (H_0: control > treatment and control < treatment) to identify treatments that were significantly different from the control. When assumptions of normality (α=0.01) and homogeneity of variance (α=0.01) were not met, nonparametric ANOVA tests (e.g., Kruskal-Wallis one-way analysis of variance or Steel's Many-One Rank Test) were used. ANOVA was followed by appropriate multiple comparison tests (e.g., Fisher's Least Significant Difference (LSD) pairwise comparison, Dunnett Multiple Comparison Test or the Bonferroni Adjusted T-Test [parametric tests]; Kruskal-Wallis All-Pairwise Comparison Test [non-parametric tests]). Statisical analyses were performed using CETIS v 1.8.7 (Tidepool Scientific 2014) or STATISTIX v 9 (Analytical Software 2008). Median lethal concentrations (LC_{50}s) and inhibition concentrations (ICs) were calculated using CETIS v 1.8.7 (2014).

3.0 RESULTS

3.1 Water Quality

Once the pH was adjusted in the NTO solutions it was very stable, with no indication of downward drift in prepared NTO solutions. In the test solutions, pH generally ranged from about 7.7 to 8.4; the highest recorded pH values were 8.5 (Table 3-1). Old solutions often had lower pHs, however, this is not unusual in toxicity tests as dissolved CO_2 from organism respiration will depress pH.

Temperatures were somewhat higher early in the study but adjustments were made to the environmental chamber control system to lower the temperatures. Regardless, the temperature range in each test treatment generally remained within the target 22 ± 3°C. Not unexpectedly, DO concentrations were often lower in the old test solutions, but never to a point that appeared to unduly stress organisms. Specific conductivity increased with NTO concentration (Table 3-1). Conductivity was higher in old solutions due to some evaporation but also because of uneaten food particulates and fecal waste.

3.2 Analytical Chemistry

The measured concentration of NTO in the control treatments was below detection in all samples except the final old sample (Table 3-2), which was collected after all organisms had either presented front limbs, and thus been transferred to emergence chambers, were removed at test termination without having developed front limbs, or died. NTO was detected in the final composite control sample at ~0.68 mg/L. Since NTO was detected in the composite sample of the final old control solutions, individual replicate control samples were submitted for analysis. The NTO concentrations in the individual replicate control samples were 0.293, 0.315, 0.139 and 0.269 mg NTO/L (mean = 0.254 mg/L). The source of the contamination in the final samples is unknown, although it is likely to have occurred at or near the end of the test, possibly during removal of the final tadpoles and/or collection of the final samples, as all earlier control samples showed no detectable NTO. Therefore, exposure of control organisms to residual NTO likely occurred for only a short period of time, if at all. However, since the mean (of the replicate samples) concentration of NTO in the final control solution (0.254 mg/L) was greater than the laboratory reporting limit of 0.0200 mg/L, 0.254 was used in the calculation of the time-weighted average NTO concentration of the control treatment for the 70-d study. The resulting NTO time-weighted average (TWA) concentration for the control treatment (0.04 mg/L) was quite low and had no impact on calculation of final statistical endpoints.

In all NTO treatments, the concentration of NTO in the old samples collected at the end of an interval was higher relative to the new samples collected at the beginning of that interval (Table

3-2). Even though test solutions were renewed weekly during the test up to day 49 (and thus the “old” samples were collected from a different batch of water than the “new” samples were), the higher measured NTO concentration in old solutions is likely reflective of evaporation of some of the test water in the chambers.

With the exception of the control treatment due to the one detectable NTO measurement at the end of the test, the percent coefficient of variation (%CV) for the NTO concentrations ranged from 1.87% in the highest NTO treatment to 16.86% in the lowest NTO treatment. Only two samples were analyzed in the highest NTO test treatment since organisms in that treatment were all dead by day 2 of the test. Those samples were taken on day 0 (new sample) and day 2 (old sample), and were 11,200 mg/L and 11,500 mg/L NTO, respectively.

Analytical data reports are presented in Appendix A.

3.2.1 Time-Weighted Average NTO Concentrations

For statistical analyses, time-weighted average (TWA) NTO concentrations were calculated. For the 14-d and 28-d metrics (test replicates E and F, respectively), only the day 0 (new solutions) and day 28 (old solutions) samples were used to calculate the TWAs. For all of the calculations made using end-of-test (70 d) data, all analytical data were included in the determination of the TWAs (Table 3-3). The TWA concentrations at the end of the test were somewhat higher than the day 28 TWAs. This was because the NTO concentrations in some of the samples collected after day 28 were higher than in the earlier samples.

3.2.2 Variability Among Replicates

Samples of old solutions from replicates A-D of two NTO treatments (3.2% and 20% of stock solution) collected on day 28 were analyzed for NTO. The analysis revealed little variability among the replicates (Table 3-4). The % CV among the replicates was about 1% for each treatment. These data suggest a high degree of consistency in exposure concentration among replicates. The mean concentrations of the individual replicates in these two treatments were also very similar to the composite concentrations of the old solutions, collected on day 28, as shown in Table 3-2.

3.3 Range-Finding Tests

At a minimum, high and low concentrations in the range-finding tests were analyzed for NTO concentration. Any intermediate concentrations for which analytical data were not available were calculated based on the percent of the high concentration.

In the tests with ambient (non-pH-adjusted) NTO, all organisms died in the high concentration (383 mg NTO/L, pH 3.7) within 24 h. Survival of younger (72 h) tadpoles was 90% or greater in all other treatments by day 7 (≥ 80% by day 9); survival of older (18 d) tadpoles was 70% or

greater by day 7 (Table 3-5). The fact that there was no statistically significant mortality in the second highest concentration (191.5 mg NTO/L) where the initial pH was 4.3 suggests that the *L. pipiens* tadpoles are relatively tolerant of somewhat acidic water. The pH in this concentration did rise over seven days to 5.4 and 4.8 in tests with the 74 h and 18 d tadpoles, respectively, with an additional rise by day 9. Longer-term exposure to sub-pH 4.5 water might have caused additional effects that were not assessed in these studies. There was no substantive difference in response between the younger and older animals. The 48-h and 7-d LC_{50}s for both age groups were around 250 mg NTO/L.

In the tests with pH-adjusted NTO, there was no statistically significant mortality in any concentration, up to 3710 mg NTO/L, making all LC_{50}s >3710 mg/L (Table 3-6). Since there was no statistically significant mortality in any concentration of pH-adjusted NTO in this range-finding test, it was decided that a higher NTO concentration needed to be included in the definitive study in order to elicit complete response.

Laboratory data sheets and printouts of statistical analyses for these studies are presented in Appendix B.

3.4 Definitive Test (14-Day and 28-Day Studies)

All organisms in the highest test concentration (11,350 mg NTO/L) were dead by day 2 of the test. Survival of *L. pipiens* was the same after 14 d and 28 d in all other test treatments. In addition to 100% mortality in the high concentration (11,350 mg/L), there was mortality in the second highest concentration (7775 mg/L) (Tables 3-7 and 3-8). SVL and body width in the 7775 mg/L NTO treatment were statistically (α=0.05) reduced relative to the control at both 14 and 28 d (Tables 3-7 and 3-8; Figures 3-1 and 3-2). At 14 d, SVL was also statistically reduced at 3070 mg/L and at the lowest concentration (201 mg/L). By 28 d, tadpoles in all treatments, except 7775 mg/L had grown to where SVLs of organisms in the NTO treatments were essentially equal to or greater than the control organisms. In the 7775 mg/L treatment, tadpoles showed an even greater difference from the control and other NTO treatments (Figure 3-1).

The relative body widths of organisms among treatments were similar at 14 and 28 d (Figure 3-2). While the control mean width was greater than in any of the NTO treatments, variability among organisms precluded detection of any statistically significant differences except at the highest NTO concentration where organisms were considerably smaller. Size differences among treatments were more visually evident at 14 d than at 28 d (Photographs 7 and 8, respectively). At 28 d, most of the tadpoles had developed hind limbs with clearly visible digits (Photograph 9), except for those in the 7775 mg/L treatment, where none of the animals had developed limbs. Though some tadpoles demonstrated a somewhat contorted vertebral column, there was no indication that this malformation was associated with NTO concentration

since some control organisms demonstrated the same abnormality while those in the highest NTO treatment did not.

Laboratory data sheets and printouts of statistical analyses for these studies are presented in Appendix C.

3.5 Definitive Test (70-Day Exposure)

All larval anurans in the highest NTO treatment (11,350 mg/L) were dead by day 2 of the test. The second highest concentration (8382 mg/L) also demonstrated statistically significant mortality early in the test, with 55%, 35% and 25% survival on days 7, 14 and 28, respectively (Table 3-9). While a few tadpoles in the 8382 mg/L treatment survived for much of the test (the last one died on day 63), they showed little or no growth and none developed hind limbs. They were relatively immobile, spending most of their time on the bottom of the test chamber.

Day 70 survival ranged from 60% (3338 mg/L) to 90% (control and 517 mg/L). All animals that survived until day 70 developed hind limbs, although some were very small and fell within the Gosner Stage 28-31 range. At least one animal in each surviving concentration (including the control) developed hind limbs but did not show front limb eruption, although some front limbs were visible under the skin.

Eighty percent (80%) of the control organisms developed front limbs and all of those completed metamorphosis (Table 3-9). A high percentage of animals in the three lowest NTO treatments (190, 517 and 1346 mg/L) also developed front limbs. Some of the animals in the 190 and 517 mg/L treatments died in the emergence chambers, so that the number completing metamorphosis in those treatments was slightly lower than the number with erupted front limbs. Only 50% of animals in the 3338 mg/L treatment completed metamorphosis by the end of the test.

Development of larval *L. pipiens* was delayed by exposure to NTO. The first tadpoles to externally-present front limbs were from the control and 517 mg/L treatments on day 40 of the test. From day 40 until near the end of the test on day 70, several larval organisms each week presented external front limbs. The minimum number of days required by any of the test organisms to reach Gosner Stage 46, (tail absorption and metamorphosis to a froglet), was 44 days. Two organisms from the control and one organism each from the 190 mg/L, 517 mg/L and 1346 mg/L treatments achieved complete metamorphosis by day 44. In the 3338 mg/L treatment, minimum days to front limb eruption and complete metamorphosis were 45 and 51, respectively.

Mean time to front limb eruption and complete metamorphosis were 46.3 and 51.0 days, respectively, in the control, and 59.2 and 61.9 days, respectively, in the 3338 mg/L treatment

(Table 3-10). There was considerable variability in the time it took for test organisms to present external front limbs and to achieve complete metamorphosis (Figure 3-3). In the control, for example, days until metamorphosis ranged from 44 to 69 while in the 3338 mg/L treatment the range was 51 to 72 days. Mean (of replicate treatments) time to front limb eruption, time to metamorphosis, and mean minimum times to each of those two metrics were always lowest in the control (Table 3-10). The 3338 mg/L treatment was always statistically greater than the control for all of these parameters. Time to front limb eruption in the control (mean = 46.3 d) was also statistically lower than the 190 mg/L (mean = 53.2 d) treatment, but not the 517 and 1346 mg/L treatments (means = 50.4 d and 49.7 d, respectively) (Table 3-10). Because the time to limb eruption was not monotonically increasing, the NOEC for all of the limb eruption and metamorphic temporal parameters was determined to be 1346 mg NTO/L.

Frogs demonstrated no overt abnormalities that were correlated with NTO concentration.

SVLs in the 517, 1346 and 3338 mg/L treatments were higher than in the control or the 190 mg/L treatments. The longest frogs were from the 3338 mg/L treatment (mean of 27.29 mm) but they were not statistically larger than the control frogs (mean of 25.85 mm) (Table 3-11). Limb lengths were very consistent among all treatment groups with no statistically significant differences from the control, with two exceptions. The left and right radio-ulna length of frogs from the 190 mg/L treatment were statistically ($p<0.05$) less than the control. However, the radio-ulna lengths of all other treatments were greater than, and not statistically different from, the control. Because these data are non-monotonic with no consistent concentration-response, these statistical differences in the 190 mg/L treatment are likely anomalous and not related to NTO concentration.

Weights of the frogs at metamorphosis in the three highest NTO treatments were greater than the control, but the differences were not statistically significant (Table 3-11). The mean weights of the control and 190 mg/L treatments were 1707 and 1703 mg, respectively, while the mean weights of the 517, 1346 and 3338 mg/L treatments were 1982, 1853 and 2030 mg, respectively. Though the increase was non-monotonic and not statistically different from the control, these data suggest the possibility that NTO is eliciting a substantive response from the test organism. The larger size of the NTO-exposed organisms was reflected in the weights of internal organs.

Dissection of the frogs revealed no lesions or obvious malformations. Organs of the digestive system, including stomach, small intestine and colon, appeared enlarged in frogs from the NTO treatments, and in particular the 3338 mg/L treatment.The mean weights of the spleen, lungs (left and right lungs weighed together), kidneys (left and right kidneys weighed together) and heart in the 3338 mg/L treatment were higher than in the control (Table 3-12). Mean weights of

the spleen and kidney in the intermediate NTO treatments also tended to be higher than the control. Lung weights in the intermediate NTO concentrations were similar to or lower than the control lung weight.

Organ biomass was normalized to the size of the frog from which they were removed by dividing by total body weight, measured when the frogs were euthanized after they reached Gosner Stage 46 (Table 3-13). Mean kidney biomass, as a proportion of body weight, was statistically higher in the 3338 mg/L treatment (0.01304) than in the control (0.00964). Normalized lung biomass in the 517 mg/L treatment was statistically less than the control. This was the only situation where normalized organ biomass was statistically less than the control and was considered an anomalous response (Table 3-13).

The NOECs, LOECs and point estimates for all of the biological parameters are listed in Table 3-14. For two of the sublethal metrics, response was either non-monotonic or was monotonic, but significant differences from the control did not occur consistently with increasing NTO concentration. Specifically, Time to Front Limb Eruption in the 190 mg/L treatment was statistically greater than in the control treatment (refer to Table 3-10). Because the next two higher NTO concentrations (517 and 1346 mg/L) were not statistically greater than the control, 1346 mg/L was selected as the NOEC, and 3338 mg/L was selected as the LOEC. In the second example, lung biomass of organisms exposed to 517 mg/L was statistically less than the control when normalized to body mass. None of the other NTO treatments showed a statistical difference, and two out of three of the other NTO treatments had lung biomass that was higher than the control. Therefore, 3338 mg/L was selected as the NOEC and 8382 mg/L was selected as the LOEC.

Laboratory data sheets and printouts of statistical analyses for these studies are presented in Appendix D.

3.5.1 Histopathology

Pathology was absent from the liver sections. Pathology was recognized in the frog renal tissue, in four of five tissues from the control and five of five tissues from the 3338 mg/L treatment. The number of granulomas (“multiple aggregates of flattened macrophages encircling necrotic cellular debris and granulocytes” see pathology report in Appendix E) was higher in the kidney samples from the 3338 mg/L NTO group, relative to the control. Also, those in the 3338 mg/L NTO exposure group were scored somewhat higher (greater effect), with four “2s” (6 to 20% affected cell type) and one “3” (21 to 40% affected cell type). Of the four control renal tissue samples that demonstrated granulomas, three were scored “1” (up to 5% affected cell type) and one was scored “2.”

In both of the control testes samples, spermatogonia were numerous. Two of the three testes collected from 3338 mg/L NTO treatment frogs contained possibly fewer spermatogonia than in the control. In one specimen from the 3338 mg/L treatment, the seminiferous tubules appeared smaller than in the control tissues. The pathology results did not provide a clear, definitive picture of the effects of NTO on testicular tissue.

The laboratory histopathology reports are presented in Appendix E.

4.0 DISCUSSION

Dissolving NTO into water causes a substantial depression of solution pH. A saturated solution of NTO in MHW has a pH of ~2.3. Under ambient conditions, without pH adjustment, NTO is acutely toxic to larval *L. pipiens* at a concentration of 383 mg/L, with an estimated LC_{50} of approximately 250 mg/L, regardless of the age of the organism at test initiation (72 h and 18 d organisms were tested). Toxicity in ambient NTO is likely associated with, or due solely to, the very low pH of the NTO solution (3.7 in the highest NTO test treatment). Mortality occurs rapidly, with little difference in survival between 48 h and 7 d. This pattern is similar to that reported by Haley et al. (2009) for *C. dubia*, where the LC_{50}s at 24 h and 48 h showed only a small difference.

Even at an initial pH of 4.3 (NTO concentration ~192 mg/L), there was no mortality of younger *L. pipiens* after 7 d and only 20% mortality of the older *L. pipiens* after 7 d. Low pH may induce multiple physiological responses from larval amphibians, however, effects on Na^+ balance have been documented and can be significant. Freda and Dunson (1984) found that, in *Rana pipiens* and two other frog species as well as larval marbled salamanders (*Ambystoma opacum*), declining pH caused an efflux of Na^+ ions, resulting in death after approximately 50% of the Na^+ had been lost. *Rana pipiens* was the most sensitive of the species tested by Freda and Dunson (1984). Some studies have shown that the negative effects of exposure to acidic water could be ameliorated by elevated external concentrations of certain cations, in particular Ca^{+2} and Mg^{+2} (Horne and Dunson 1995). Dilution itself can help neutralize the pH of a NTO solution accidentally released to the environment, particularly if dilution increases with a down gradient surface plume of water. However, concentrated NTO that is spatially confined could cause rapid acute toxicity to resident organisms. A comparison of the current toxicity data on *L. pipiens* with the *C. dubia* data of Haley et al. (2009) suggests that the northern leopard frog is not the most sensitive species that may be present in any given aquatic ecosystem. The sensitivity of *C. dubia* to a variety of toxicants is well documented, making it a preferred invertebrate test species for whole effluent toxicity (WET) tests (Grothe et al. 1996).

The long-term studies described here were conducted with pH-adjusted NTO. Once adjusted, there was essentially no downward pH drift in the NTO stock solution, and the pH of test treatments was stable. These results indicate that pH-adjusted NTO is acutely toxic to *L. pipiens* only at very high concentrations. At an NTO concentration exceeding 11,000 mg/L, mortality occurred rapidly, with all organisms dead by day 2. In the next lowest concentration (8382 mg/L) mortality also occurred quickly but some organisms remained alive for up to nine weeks in the test system. The surviving organisms, however, did not thrive but remained undeveloped (no limbs) and very lethargic, spending most of their time on the bottom of the test chambers. The last remaining test organism in the 8382 mg/L treatment died on day 63. While survival of *L.*

pipiens was not statistically reduced in any of the remaining NTO concentrations, it was noticeably lower at 3338 mg/L (60% survival at 70 d). The survival endpoints (NOEC and LOEC) reported here are substantially higher than those reported by Stanley et al. (2015) in a 28-d chronic exposure study. They reported a LOEC of 5 mg NTO/L and a NOEC of 1.5 mg/L. While survival (~68%, by extrapolation from a published chart) in the 5 mg/L treatment was statistically lower than the control, survival did not montonically decrease with increasing NTO concentration. Survival of *L. pipiens* in the 100 mg NTO/L treatment, for example, was about the same or a little higher than in the 5 mg/L treatment. The 5 mg/L LOEC, therefore, may overestimate the toxicity of NTO to *L. pipiens*. While some test methods used by Stanley et al. (2015) were different than those of the current study, it is unknown whether the differences might have impacted test results.

The 14-d and 28-d growth data suggest growth of young *L. pipiens* is significantly impacted by NTO. However, by day 28, NTO-exposed tadpoles had somewhat “caught up” with the control organisms, with the exception of those in the high (7775 mg/L) treatment where tadpoles remained clearly smaller than the controls. It was in this NTO treatment that all organisms were dead by day 63 of the definitive study.

Lotufo et al. (2015) reported that NTO did not bioaccumulate in *L. pipiens* following short-term exposure. *Lithobates pipiens* tadpoles were exposed to 100 mg/L of pH-adjusted (to pH 7.5) NTO for 24 h. The body residue NTO concentration was 25 mg/kg, resulting in a BCF of 0.25 L/kg, indicating substantial NTO dilution within tissues and very low tendency for NTO to accumulate. These data suggest that, although NTO can cause toxicity at very high concentrations, significant bioaccumulation is unlikely, and release of NTO from the body could be prominent, should juvenile animals move from water containing NTO to water that is NTO-free.

The apparent effects on growth in young *L. pipiens* tadpoles (refer to the 14-d growth data, Table 3-7) may be reflected in the increased time required for larval organisms to present front limbs and complete metamorphosis. The increasing amount of time needed for larval frogs to complete metamorphosis has ecological implications, where survival to maturity might be significantly decreased if the time spent in an ephemeral pool is increased by only a few days. Larval anurans developing in shrinking ponds may die if all the water evaporates or is drained from the basin (in the case of irrigation supply reservoirs) before animals have a chance to emerge as adults. In addition, extended time in juvenile form might increase the likelihood of predation from fish, birds, reptiles or other aquatic predators since escape out of the water is not an option. Reduced adult fitness may also result from less time to feed in the terrestrial environment and build much-needed fat reserves before winter sets in (James et al. 2005). A

reduced pool of breeding adults could lead to lower reproduction and emergence the following year.

The absolute weight of various organs increased with increasing NTO concentrations. When normalized to the mass of the whole frog, however, there were few significant differences. Only kidney biomass in the 3338 mg /L NTO treatment was statistically greater than the control. Crouse et al. (2015) reported that kidney mass in rats exposed by gavage to 1500 mg/kg-d of NTO was significantly elevated, relative to the control group. Crouse et al. (2015) also reported a statistically significant increase in the brain mass of female rats exposed to NTO at doses of 100 and 1000 mg/kg-d. Females in an intermittent treatment group (315 mg/kg-d) also had larger, but not significantly larger, brain mass than females in the control group.

The mass of testes and epididymides was significantly reduced in male rats at NTO doses as low as 30 mg NTO/kg-d in a 90-d study (Crouse et al. 2015). Microscopic examination of reproductive tissues from male rats in the 315 and 1000 mg/kg/d dose groups demonstrated moderate to severe degeneration and atrophy of the seminiferous tubules. Interestingly, the tubules were more severely atrophied in the 315 mg/kg-d group than in the 1000 mg/kg-d group, with no spermatic precursor cells or adult sperm (Crouse et al. 2015). In the current study, there were fewer, possibly less robust spermatogonia in some specimens from the 3338 mg/L NTO-exposure group, although data were inadequate to draw definitive conclusions regarding the impact of NTO on the *L. pipiens* male reproductive system.

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5.0 CONCLUSIONS

- Dissolution of NTO into an aqueous media causes a substantial drop in pH; under ambient pH conditions, acute toxicity of a NTO solution to *L. pipiens* is substantially greater than when the acidic pH is neutralized pior to testing.
- A pH-adjusted NTO solution is highly stable and can be stored for an extended period of time with essentially no pH drift.
- NTO concentrations tended to be higher in the analytical samples collected at the end of a renewal period ("old" solutions), which is likely due to evaporation from the test chamber; however, overall NTO concentrations were stable over time.
- Data on two test treatments indicate that inter-replicate variability of NTO concentrations was low. In the 28-d old solution samples collected from replicates A-D of two of the test treatments, the mean concentrations of 514 and 3285 mg NTO/L yielded %CVs of only 1.12 and 0.91, respectively.
- NTO in neutralized (pH-adjusted) solutions caused statistically significant mortality to *L. pipiens* at concentrations of 8382 mg/L and greater in a 70-d exposure. While mortality at 3338 mg/L was not shown to be statistically different from the control, reduced survival at test termination (60%) in this concentration may be ecologically significant, and suggests that statistically significant impacts might be identifiable if a larger sample size is used in future testing.
- NTO caused no obvious abnormalities or malformations in the test organisms exposed up to, and including, 3338 mg NTO/L.
- Exposure to NTO increased the amount of time required for front limb eruption and completion of metamorphosis, although the effects on limb eruption and metamorphosis were only statistically significant at 3338 mg/L. Delay in metamorphosis and emergence from a lentic waterbody could increase the likelihood of death and/or reduced adult fitness with implications for long-term survival and breeding success.
- Weight of internal organs increased with NTO exposure. When normalized to body mass, only kidney biomass from frogs in the 3338 mg/L treatment was statistically higher than the control.
- The lowest NOEC calculated in the current study was 1346 mg NTO/L for the following parameters: Time to Front Limb Eruption, Minimum Time to Front Limb Eruption, Time to Metamorphosis, Minimum Time to Metamorphosis and Kidney Biomass.
- No pathology was evident in hepatic tissues but pathology was identified in the renal tissues, with a higher number of granulomas present in animals from the 3338 mg/L NTO treatment group.

- The effect of NTO on the *L. pipiens* reproductive system in unclear. Although some NTO-exposed animals demonstrated fewer, less robust spermatogonia, there are insufficient observations to draw definitive conclusions.

6.0 ACKNOWLEDGEMENTS

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TABLES

Table 2-1. Characterization of Moderately Hard Water (MHW) used as Dilution/Control Water

Parameter	MHW Batch Number						
	11644	11658	11668	11675	11692	11702	11727
Hardness (mg/L $CaCO_3$)	90	88	86	88	90	90	86
Alkalinity (mg/L $CaCO_3$)	61	58	57	61	59	58	58
TRC (mg/L)[a]	0.02	0.02	<0.02	<0.02	<0.02	<0.02	0.03
NH_3-N (mg/L)[b]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

[a] Total Residual Chlorine
[b] Ammonia as Nitrogen

Table 2-2. Feeding Levels and Schedule

Amount of Food (Ground Tetrafin) per Test Chamber (g)[a]	Feeding Schedule[b]	Notes[c]
0.025	First feeding on Day 2	½ a “Nip”
0.045	Second feeding on Day 5	“Nip”
0.077	Days 7 to Day 12	“Smidgen”
0.1865	Days 14 – Day 26	“Pinch”
0.3055	Day 28 until test termination	“Dash”

[a] If there was ≥ 50% mortality in any test chamber, the amount of food was reduced to the next smallest amount shown in this table. This is reflective of the practice in EPA Test Method 1000.0 (USEPA 2002).
[b] Test chambers were fed on Monday, Wednesday and Friday.
[c] “Name” of spoon as designated by manufacturer; smallest was a “Nip”; largest was a “Dash.”.

Table 3-1. Water Quality Measurements (Minimum to Maximum) during the 70-d Definitive Study

NTO T-W Avg. Concn. (mg/L)	pH	Temperature (°C)	D.O. (mg/L)	Conductivity (µS/cm)
0.04	7.8 - 8.4	20.2 - 25.1	4.6 - 6.8	294 - 621
190	7.7 - 8.5	20.2 - 25.3	4.5 - 6.8	396 - 762
517	7.8 - 8.5	20.2 - 24.5	3.4 - 6.8	548 - 873
1346	7.7 - 8.4	20.2 - 24.6	4.1 - 6.8	887 - 1684
3338	7.7 - 8.3	20.3 - 25.0	3.8 - 6.8	1739 - 2584
8382	7.7 - 8.3	20.4 - 24.9	4.9 - 6.9	3760 - 5480
11,350	7.6 - 7.8	20.4 - 23.7	6.3 - 6.7	5560[a]

[a] Measurements in the 11,350 mg/L treatment taken only on day 0 (initiation) and day 2 (all organisms were dead on day 2); conductivity was unchanged between the two days.

Table 3-2. Concentration of NTO in Analytical Samples

Treatment (% of NTO Stock)	NTO Concentration (mg/L)[a]							Mean	Std. Dev.	% CV
Test Day	0	2	28	28	49	49	70			
Type[b]	New	Old	Old	New	Old	New	Old			
0	0	na	0	0	0	0	0.254[c]	0.042	0.104	244.95
1.28	191	na	211	210	220	145	154	188	31.78	16.86
3.2	445	na	514	526	556	532	549	520	39.94	7.68
8	1120	na	1340	1300	1390	1330	1670	1358	178.60	13.15
20	2880	na	3260	3280	3620	3400	3770	3368	311.02	9.23
50	7150	na	8400	8310	9180	8440	9220	8450	753.39	8.92
75	11,200	11,500	na	na	na	na	na	11,350	212.13	1.87

[a] All NTO measurements shown here were reported by USACE-ERDC-EP-C in reports included in Appendix A.
[b] 'New' solutions were freshly prepared mixtures to be used for renewal; 'Old' solutions were those from the previous renewal (in the test chambers for at least one week).
[c] Mean of individual replicate concentrations.

Table 3-3. Time-Weighted Average (TWA) NTO Concentrations

Treatment (% of NTO Stock)	≤ 28 d TWA Concentrations (mg/L)	70+ d TWA Concentrations (mg/L)
0	0	0.04
1.28	201	190
3.2	480	517
8	1230	1346
20	3070	3338
50	7775	8382
75	11,350[a]	11,350[a]

[a] Only one new and one old sample were collected from the highest concentration, therefore the TWA NTO concentrations for the ≤ 28-d calculations and the 70+ d calculations were the same.

Table 3-4. Variation in NTO Concentrations among Replicates

Treatment (% of NTO Stock)	NTO Concentration in Individual Replicates (mg/L)[a]				Mean (mg/L)	Std. Dev.	% CV
	Rep A	Rep B	Rep C	Rep D			
1.28	517	506	519	513	513.75	5.74	1.12
20	3300	3320	3260	3260	3285	30	0.91

[a] Old solution samples collected on day 28.

Table 3-5. Results of Range-Finding Tests using Ambient (non-pH adjusted) NTO Solutions

NTO Concn. (mg/L)	Init. pH	Younger Tadpoles (~72 h old)			Older Tadpoles (18 d old)		
		Day 7 Survival (%)	48-h LC_{50} (mg NTO/L)	7-d LC_{50} (mg NTO/L)	Day 7 Survival (%)	48-h LC_{50} (mg NTO/L)	7-d LC_{50} (mg NTO/L)
0	8.2	100	270.8	252.8	70	235.8	255.2
24.5	7.4	100			100		
47.9	6.9	90			90		
95.8	6.3	100			90		
191.5	4.3	100			80		
383	3.7	0			0		

Table 3-6. Results of Range-Finding Tests using Neutralized (pH-adjusted) NTO Solutions

NTO Concn. (mg/L)	Init. pH	Younger Tadpoles (~72 h old)			Older Tadpoles (18 d old)		
		Day 7 Survival (%)	48-h LC_{50} (mg NTO/L)	7-d LC_{50} (mg NTO/L)	Day 7 Survival (%)	48-h LC_{50} (mg NTO/L)	7-d LC_{50} (mg NTO/L)
0	8.1	90	>3710	>3710	90	>3710	>3710
943	7.9	90			80		
1840	7.7	90			80		
3710	7.4	90			100		

Table 3-7. Survival and Growth of Tadpoles after 14-d Exposure to NTO

NTO T-W Avg. Concn. (mg/L)	Survival (%)[a]	SVL			Body Width		
		Mean SVL (mm)[a]	14-d IC_{25} (mg /L)	14-d IC_{10} (mg/L)	Mean Body Width (mm)[d]	14-d IC_{25} (mg/L)	14-d IC_{10} (mg/L)
0	100	14.0			7.6		
201	100	11.6[b]			6.3		
480	80	12.8			7.1		
1230	80	12.2	6295	61	7.1	6059	1307
3070	100	12.2[b]			6.7		
7775	60	10.0[b]			5.3[b]		
11,350	0	na			na		

[a] 14-d data based on organisms from a single replicate (E).
[b] Growth is significantly (α=0.05) reduced relative to the control.

Table 3-8. Survival and Growth of Tadpoles after 28-d Exposure to NTO

NTO T-W Avg. Concn. (mg/L)	Survival (%)	SVL			Body Width		
		Mean SVL (mm)	28-d IC_{25} (mg /L)	28-d IC_{10} (mg/L)	Mean Body Width (mm)	28-d IC_{25} (mg/L)	28-d IC_{10} (mg/L)
0	100	22.8			13.0		
201	100	22.6			10.6		
480	80	22.0			11.0		
1230	80	23.8	6044	4025	11.2	4350	28
3070	100	23.0			10.8		
7775	60	15.0[b]			8.0[b]		
11,350	0	na			na		

[a] 28-d data based on organisms from a single replicate (F).
[b] Growth is significantly (α=0.05) reduced relative to the control.

Table 3-9. Summary of Survival, Front Limb Eruption, and Metamorphosis in the 70-d Definitive Study[a]

NTO T-W Avg. Concn. (mg/L)	14-d Surv. (%)	28-d Surv. (%)	70-d Surv. (%)	Live Organisms w Front Limbs (% of orig)	Organisms Completing Metamorphosis (% of orig)
0.04	100 (0)	95 (5.0)	90 (5.8)	80 (8.2)	80 (8.2)
190	100 (0)	100 (0)	80 (11.6)	75 (9.6)	65 (12.6)
517	100 (0)	100 (0)	90 (10.0)	70 (5.8)	65 (5.0)
1346	95 (5.0)	95 (5.0)	80 (14.1)	75 (12.6)	75 (12.6)
3338	85 (5.0)	85 (5.0)	60 (0)	55 (5.0)	50 (5.8)
8382	35 (5.0)[b]	25 (5.0)[b]	0[b]	---	---
11,350	0[b]	0[b]	0[b]	---	---

[a] Mean (S.E.) values are shown.
[b] Significantly (α=0.05) reduced relative to the control.

Table 3-10. Summary of Time to Front Limb Eruption and Metamorphosis in the 70-d Definitive Study[a]

NTO T-W Avg. Concn. (mg/L)	Time to Front Limb Erupt. (Days)	Min. Time to Front Limb Erupt. (Days)[b]	Time to Metamorphosis (Days)	Min. Time to Metamorphosis (Days)[b]
0.04	46.3 (1.8)	40.8 (0.5)	51.0 (2.0)	45.2 (0.8)
190	53.2 (0.8)[c]	44.8 (1.3)	55.8 (0.6)	49.2 (1.8)
517	50.4 (1.7)	43.0 (1.3)	53.5 (0.7)	47.8 (1.8)
1346	49.7 (2.0)	43.5 (1.6)	54.6 (2.2)	48.0 (2.0)
3338	59.2 (2.3)[c]	52.0 (2.7)[c]	61.9 (2.5)[c]	58.0 (2.5)[c]

[a] Mean (S.E.) values are shown.
[b] Minimum time to front limb eruption and to metamorphosis represents the time it took the first organism in the treatment to reach the respective endpoint.
[c] Significantly (α=0.05) higher than the control.

Table 3-11. Summary of Weight, SVL and Limb Lengths of Frogs upon Completion of Metamorphosis (Gosner Stage 46)[a]

NTO T-W Avg. Concn. (mg/L)	Wet Wt. at Metamorphosis (mg)	Length (mm)						
		SVL	Radio-ulna		Femur		Tibio-fibula	
			Left	Right	Left	Right	Left	Right
0.04	1707 (64.5)	25.85 (0.197)	5.88 (0.112)	5.87 (0.091)	11.27 (0.149)	11.21 (0.096)	12.48 (0.203)	12.50 (0.213)
190	1703 (165.2)	24.43 (1.160)	4.97[b] (0.270)	5.08[b] (0.381)	10.26 (0.783)	10.01 (0.580)	11.06 (1.054)	11.18 (0.746)
517	1982 (144.7)	26.66 (0.733)	5.52 (0.088)	5.94 (0.117)	11.42 (0.448)	11.23 (0.624)	12.83 (0.429)	12.74 (0.577)
1346	1853 (243.0)	26.34 (1.128)	5.52 (0.172)	5.91 (0.228)	11.03 (0.750)	10.76 (0.819)	12.24 (0.650)	12.20 (0.783)
3338	2030 (48.8)	27.29 (0.313)	5.71 (0.062)	5.92 (0.131)	11.56 (0.148)	11.17 (0.150)	12.43 (0.304)	12.23 (0.196)

[a] Mean (S.E.) values are shown.
[b] Significantly (α = 0.05) reduced relative to the control.
Note: All mean values are per surviving number of organisms.

Table 3-12. Summary of Organ Weights from Frogs Completing Metamorphosis (Gosner Stage 46)[a]

NTO T-W Avg. Concn. (mg/L)	Blot Dry Weight per Surviving Organism (mg)[b]				
	Spleen	Kidney	Lung	Liver	Heart
0.04	0.634 (0.100)	16.11 (1.294)	14.16 (2.684)	66.26 (3.100)	9.889 (0.736)
190	0.681 (0.078)	17.36 (0.514)	14.93 (2.286)	64.87 (8.467)	9.538 (0.721)
517	0.908 (0.095)	17.67 (0.856)	9.650 (0.721)	91.69 (7.243)	12.75 (0.728)
1346	0.682 (0.145)	15.82 (0.247)	11.84 (1.209)	71.40 (4.984)	10.65 (0.545)
3338	1.189 (0.091)	25.52 (2.604)	22.93 (2.261)	74.09 (5.113)	13.14 (0.327)

[a] Mean (S.E.) values are shown.
[b] Organs were blotted with laboratory paper to remove excess moisture before being weighed.

Table 3-13. Organ Biomass (as a Proportion of Body Weight)[a]

NTO T-W Avg. Concn. (mg/L)	Spleen	Kidney	Lung	Liver	Heart
0.04	0.00039	0.00964	0.00841	0.03967	0.00612
190	0.00046	0.01131	0.00967	0.04079	0.00621
517	0.00042	0.00836	0.00454[b]	0.04237	0.00598
1346	0.00042	0.00984	0.00742	0.04374	0.00657
3338	0.00060	0.01304[c]	0.01170	0.03716	0.00672

[a] Mean values are shown.
[b] Significantly (α=0.05) less than the control.
[c] Signfiicantly (α=0.05) greater than the control.

Table 3-14. Summary of Statistically-Determined Endpoints in the Definitive Test

Parameter	Endpoint (mg/L NTO)				
	NOEC	LOEC	LC_{50} (95% C.L.)	IC_{25} (95% C.L.)	IC_{10} (95% C.L.)
14-d Survival	3070	7775	5040 (3890-6366)	---	---
28-d Survival	3070	7775	5279 (4014-6449)	---	---
70-d Survival	3338	8382	3670 (2798-4814)	---	---
No. with Front Limb Eruption	3338	8382	---	2575 (na-4313)	1381 (na-2599)
No. Completing Metamorphosis	3338	8382	---	1999 (na-4369)	30.04 (na-3079)
Time to Front Limb Eruption[a]	1346	3338	---	---	---
Min. Time to Front Limb Eruption	1346	3338	---	---	---
Time to Metamorphosis	1346	3338	---	---	---
Min. Time to Metamorphosis	1346	3338	---	---	---
Frog Wt. at Metamorphosis	3338	8382	---	>3338	>3338
SVL of Frogs	3338	8382	---	>3338	>3338
Radio-Ulna Length (Right)	3338	8382	---	>3338	>3338
Radio-Ulna Length (Left)	3338	8382	---	>3338	>3338
Femur Length (Right)	3338	8382	---	>3338	>3338
Femur Length (Left)	3338	8382	---	>3338	>3338
Tibio-Fibula Length (Right)	3338	8382	---	>3338	>3338
Tibio-Fibula Length (Left)	3338	8382	---	>3338	>3338
Liver Biomass	3338	8382	---	---	---
Heart Biomass	3338	8382	---	---	---
Kidney Biomass	1346	3338	---	---	---
Lung Biomass[a]	3338	8382	---	---	---
Spleen Biomass	3338	8382	---	---	---

[a] These data sets demonstrated interrupted statistical significance, with some higher NTO concentrations being non-significant; the higher concentration showing no statistical significance was selected as the NOEC.

FIGURES

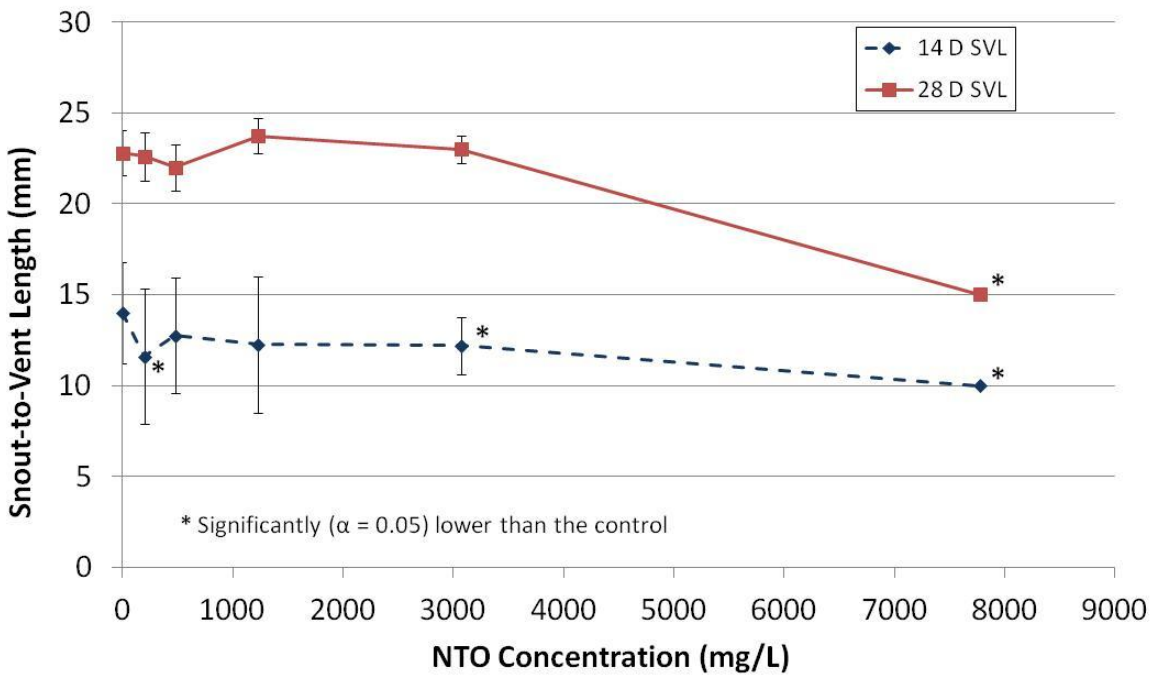


Figure 3-1. Snout-to-Vent Length (SVL) (Mean ± 1 Std. Dev.) of *L. pipiens* Tadpoles at Test Days 14 and 28

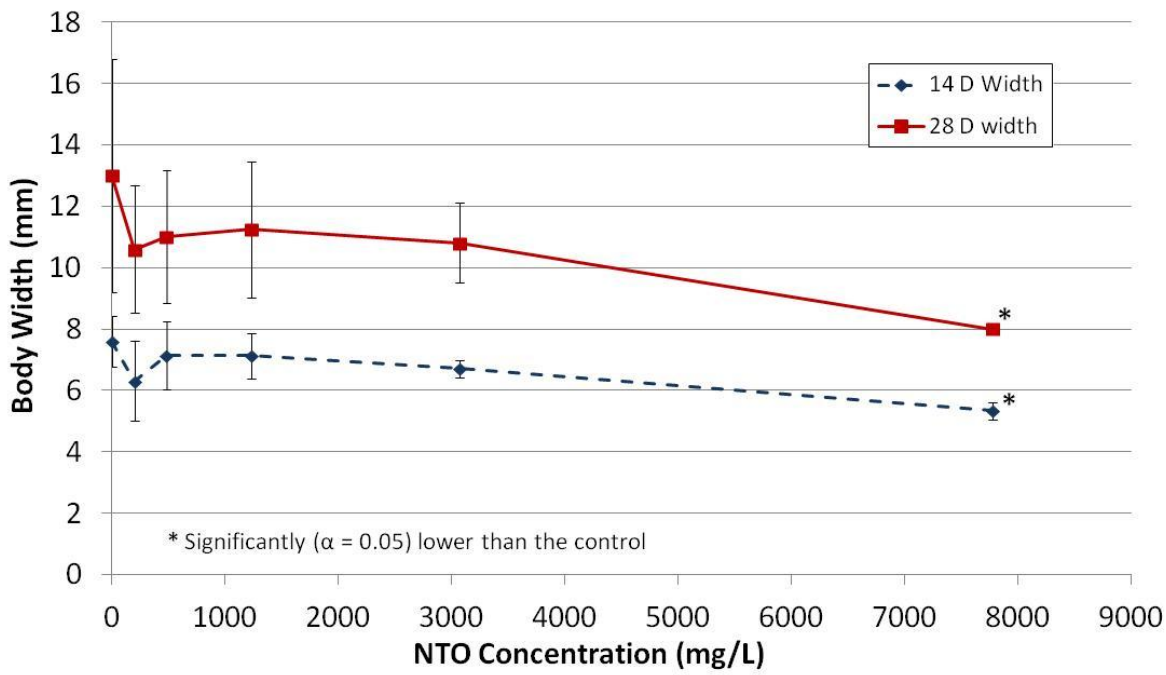


Figure 3-2. Body Width (Mean ± 1 Std. Dev.) of *L. pipiens* Tadpoles at Test Days 14 and 28

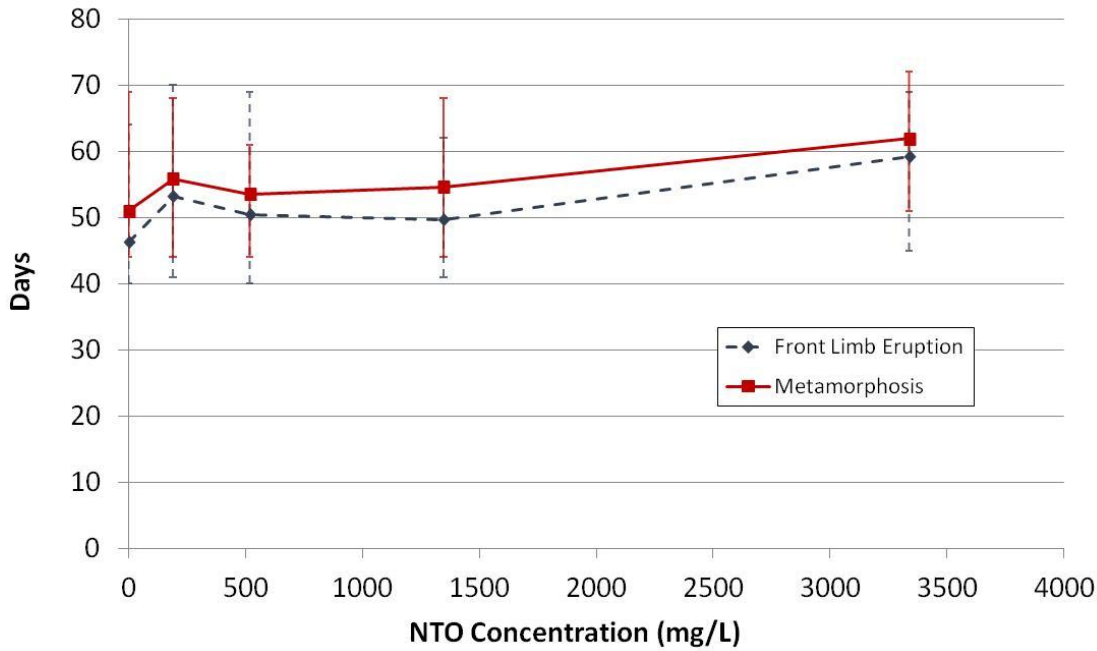
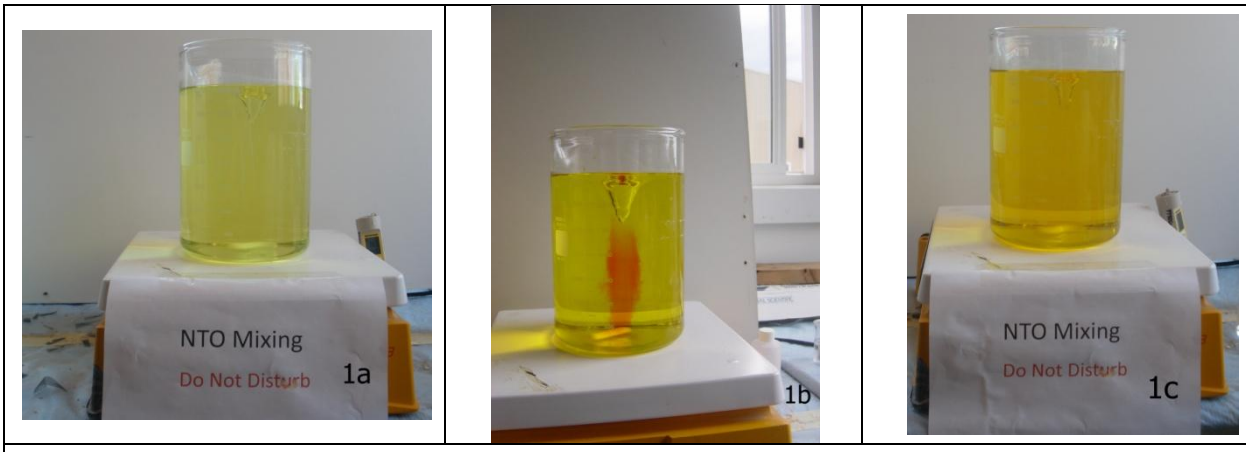
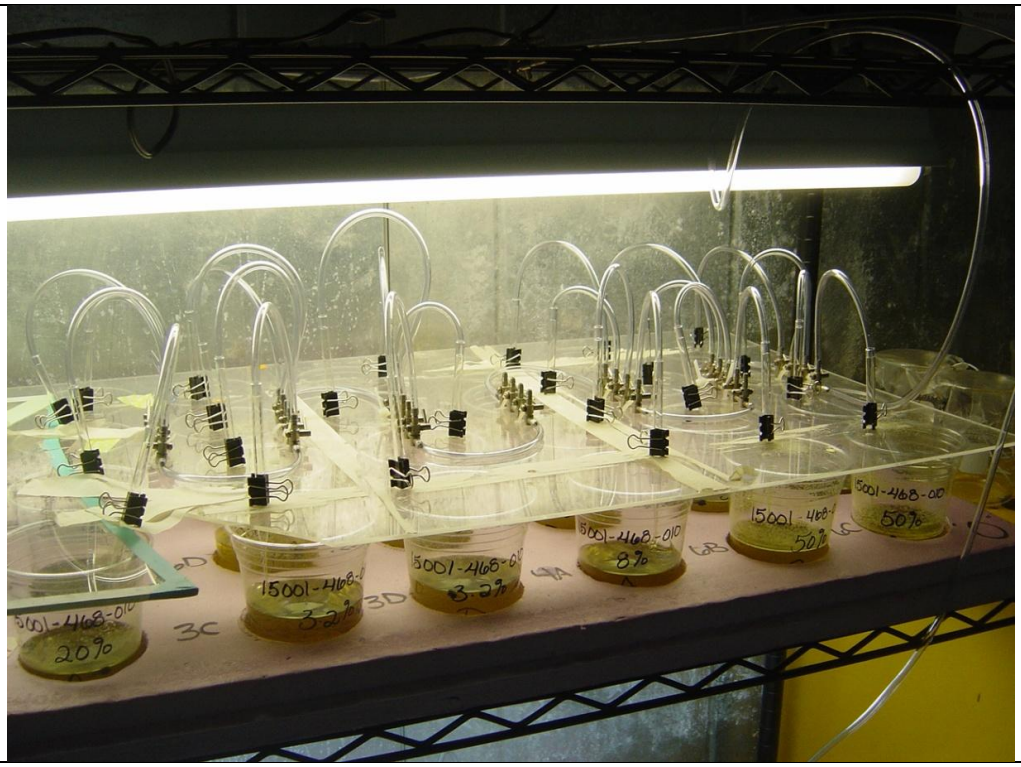


Figure 3-3. Comparison of Mean Days Until Front Limb Eruption and Metamorphosis. Error Bars Display Range for that Treatment

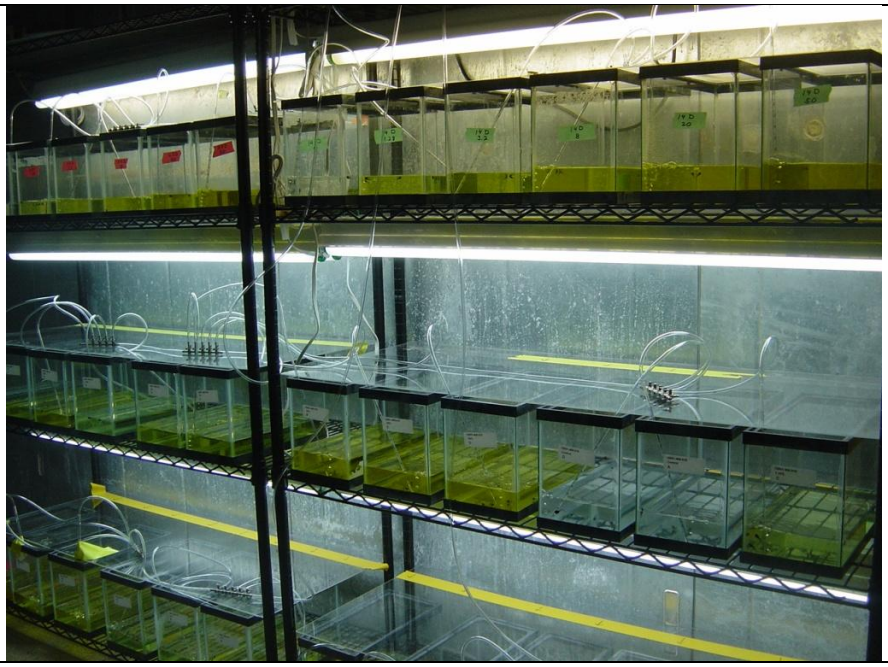
PHOTOGRAPHS



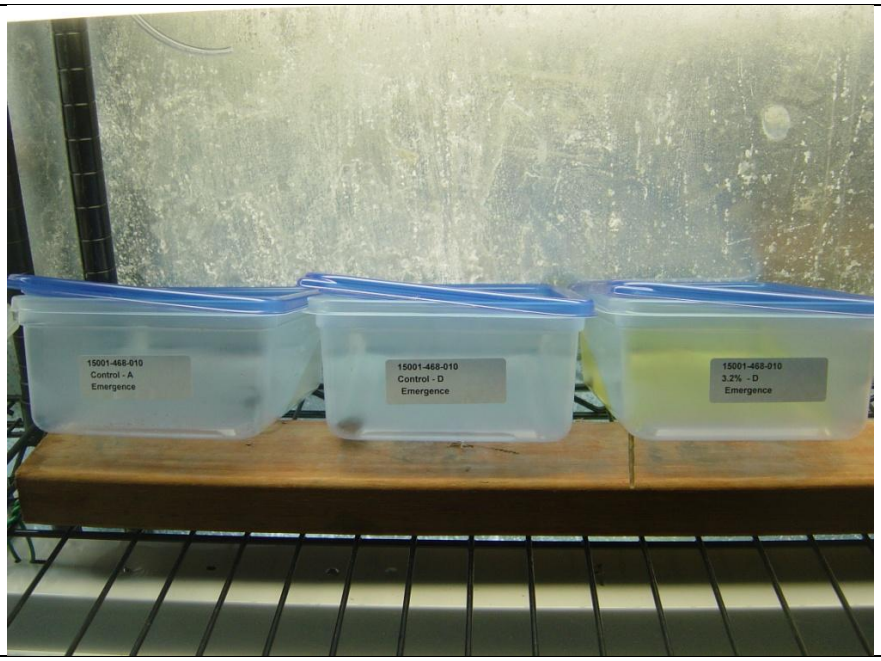
Photograph 1. NTO in MHW before (1a), during (1b) and after (1c) addition of NaOH to raise the pH from ~2.3 to ~7.5.



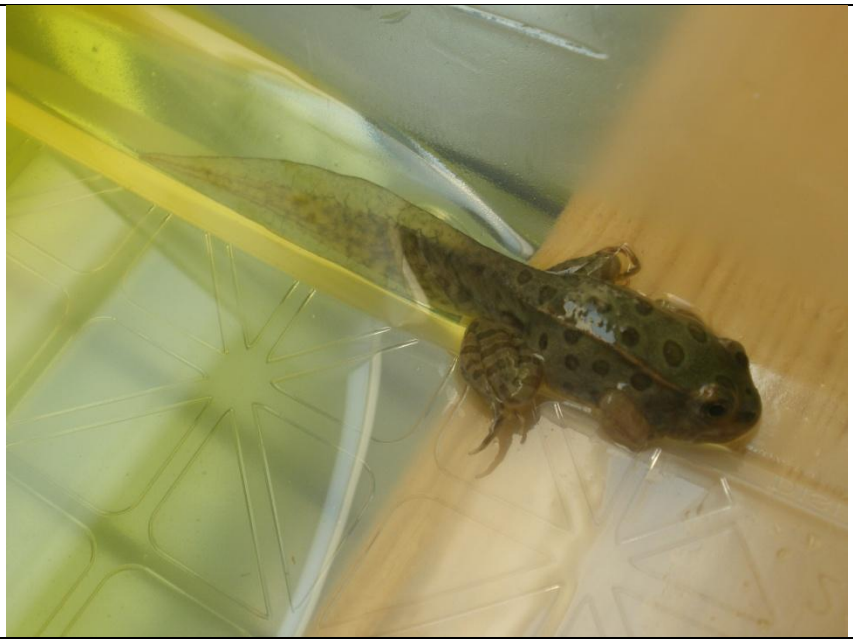
Photograph 2. Test exposure design at test initiation. Each exposure cup is randomized on the board.



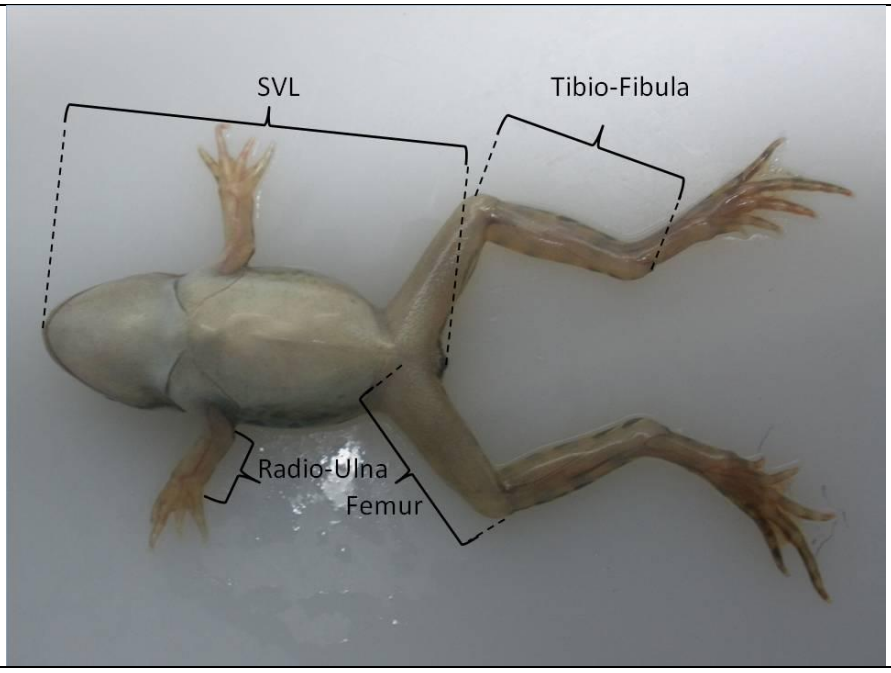
Photograph 3. Exposure chambers from day 7 to end of the test. Chamber positions are randomized on the shelves.



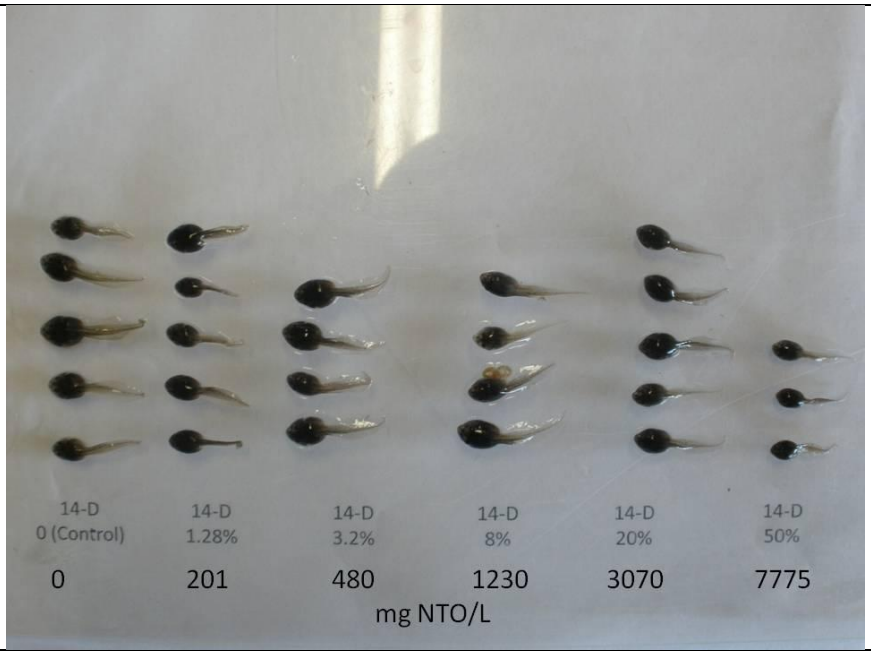
Photograph 4. Emergence chambers.



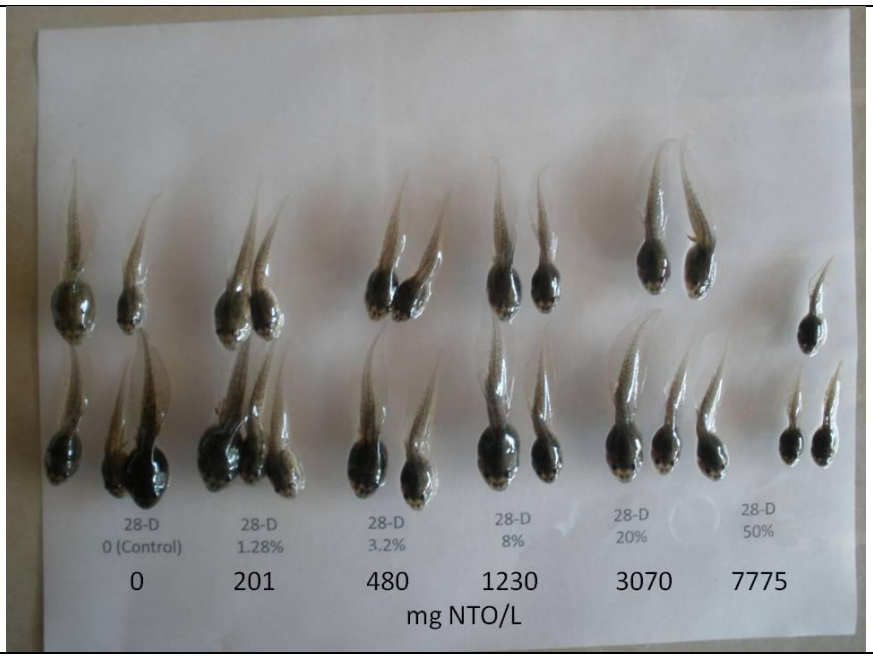
Photograph 5. Tadpole with front limbs in emergence chamber; resting on dry surface.



Photograph 6. Length measurements taken on frogs after metamorphosis.



Photograph 7. Euthanized tadpoles after 14-d exposure to NTO.



Photograph 8. Euthanized tadpoles after 28-d exposure to NTO.

Photograph 9. Closeup of hind limb on tadpole exposed to 1230 mg NTO/L for 28 days.

US Army Public Health Center
Aberdeen Proving Ground, MD

Study Contract Title: One Generation Amphibian Study
W91ZLK-14-P-0372

APPENDIX B

RANGE-FINDING TESTS
DATA PACKAGES & STATISTICAL ANALYSES

Prepared by

TRE Environmental Strategies

TRE Environmental Strategies, LLC
Fort Collins, CO

March 2016

TRE Project Number: 15001-468-710

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Acute

Project Number: 15001-~~2~~68-006 40 ①

Test Substance: NTO - Unadjusted (ambient pH)

Species: ~~Rana~~ Lithobates pipiens ①

Dilution Water: Mod Hard

Organism Lot or Batch Number: 15-012

Concurrent Control Water: NA

Age: ~72 h (24-72 h)

Supplier: Carolina Biological

Date and Time Test Began: 3/25/15 @ 1200

Date and Time Test Ended: 4/3/15 @ 0850

Protocol Number:

Investigator(s): DAP/SP/A/MW

Background Information

Type of Test: Static-~~Renewal~~ ①

pH control?: Yes No

If yes, give % CO_2: NA

Test Temperature: 25 ± 1 °C

Env. Chmbr/Bath #: 1

Test Chmbrs: 500-ml cups/~~beakers~~ ②

Photoperiod: 16 h light : 8 h dark

Light intensity: 50-100 ft-c.

Test Solution Vol.: 250 ml

Replicates per Treatment: 2

Length of Test: ① 7 ~~14~~-21 days

Organisms per Replicate: 5

Type of Food and Quantity per Chamber: Grnd Tetramin; ~ 0.2 g

Feeding Frequency: ~ every ~~2~~ ① ~~3~~ 2 days

often Gosner stage 25

Test Substance Characterization Parameters and Frequency:

Hardness:

Alkalinity:

NH_3:

TRC:

pH: Init and Term

Conductivity: Init and Term

Test Concentrations (Volume:Volume): MH, 0.31, 0.625, 1.25, 2.5, and 5% NTO Saturated Stock
(5% is considered 100% for remainder of data pacakge)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: _____ to _____ IC_{25}: _____

Hist. 95% Control Limits: _____ to _____ Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Study Director Initials: DAP Date: 3/25/15

① DAP 10/6/15 E ② DAP 10/12/15 - Plastic cups were used

QA: AR 10/9/15

TEST SUBSTANCE USAGE LOG

Project Number: 15001-~~2~~4 ② 68-006

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: @ NA	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (Grab or Comp)	NA			
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	11553			
Concurrent Control Water RW#	NA			
Date(s) Used	3/25/15			

Preparation of Test Solutions

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	500	500	0	1000	1000			
6.25%	31.25	468.75	500	63 ② ①	938	1000			
12.5%	62.5	437.5	500	125	875	1000			
25%	125	375	500	250	750	1000			
50%	250	250	500	500	500	1000			
100%	500	0	500	1000	0	1000			
						①			
				①		~~6000~~			
Total	968.75	2031.25	3000	1938 ①	4063	~~5000~~			

Initials / Date	~~3/25/15 DSP~~ ①	3/25/15 DSP	
Initials / Date			
Initials / Date		Note: 1000 ml prepared	
Initials / Date		for Tests 006 and	
Initials / Date		007. DSP 3/25/15	
Initials / Date			
Initials / Date			
Initials / Date			

① DSP 3/25/15 E ② DSP 10/6/15 E

QA Form No. 060
Revision 3
Effective 02/14

(3) ~~(2)~~ Northern Leopard Frog (Lithobates p'piens)

~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

QA: AR 10/9/15

Project Number: 15001-~~2~~4 ~~(2)~~ (3) 68-006

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
MH	A	5	5	5	5	5	5	5	5	
NTO	B	5	5	5	5	5	5	5	5	
= 0	C	~~5~~	~~(1)~~							
	D	~~5~~	~~(1)~~							
6.25%	A	5	5	5	5	5	5	5	5	
NTO=	B	5	5	5	5	5	5	5	5	
(4) ~~24.5~~	C	~~5~~	~~(1)~~							
23.7 24.5 ~~24.1~~ (3)	D	~~5~~	~~(1)~~							
12.5%	A	5	5	5	5	5	5	5	4	
NTO	B	5	5	5	5	5	5	5	5	
=47.9	C	~~5~~	~~(1)~~							
	D	~~5~~	~~(1)~~							
25%	A	5	5	5	5	5	5	5	5	
NTO=	B	5	5	5	5	5	5	5	5	
95.8	C	~~5~~	~~(1)~~							
	D	~~5~~	~~(1)~~							
50.0%	A	5	5	5	5	5	5	5	5 [A1]	A1 Food from day 5 still present, D ~~oit appear to be~~ eating.
NTO=	B	5	5	5	5	5	5	5	5 [A1]	
191.5	C	~~5~~	~~(1)~~							
	D	~~5~~	~~(1)~~							
100.0%	A	5	0	0	—	—	—	—	—	
NTO=	B	5	0	0	—	—	—	—	—	
383	C	~~5~~	~~(1)~~							
	D	~~5~~	~~(1)~~							
	A									
	B									
	C									
	D									
Date:		3/25/15	3/26/15	3/27/15	3/28/15	3/29/15	3/30/15	3/31/15	4/1/15	
Time:		1200	1710	0815	1250	1335	0915	1430	0920 ~~0920~~	
Initials:		DAP	DAP	SP	AN	MW	SP	DAP	SP	

(1) DAP 3/25/15 E - only 2 reps
(2) SP 4/1/15 E - Date is 4/1/15, time is 0920
(3) DAP 10/6/15 E
~~(5)~~ (4) DAP 10/12/15 E; measured concn. used.

① Northern Leopard Frog (Lithobates pipiens)

~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

QA: ARC 10/9/15

Project Number: 15001-268-006 ①

%Conc.	Test Replicate	Number of Surviving Organisms Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Remarks
MH	A	5	5							
	B	5	5							
	C									
	D									
6.25%	A	5	5							
	B	5	5							
	C									
	D									
12.5%	A	3	3							
	B	5	5							
	C									
	D									
25%	A	5	5							
	B	5	5							
	C									
	D									
50.0%	A	5	5							
	B	5	5							
	C									
	D									
100.0%	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
Date:		4/2/15	4/3/15							
Time:		1650	0850							
Initials:		DAP	SP							

① DAP 10/6/15 E

② Am 11/5/15 E

QA: AR10/9/15

CHRONIC CHEMICAL DATA (~~INITIAL~~ ALL) ①

Project Number: 15001-~~2~~4 ① 68-006

Test Species: ~~Rana~~ Lithobates ① pipiens

%	Day 0 (New)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: MH									All Conc.	
pH	8.2	8.4	8.4						FM23	
D.O. (mg/L)	7.2	7.0	6.6						5	
Temp. (°C)	19.1								L-5	
Cond. (µS/cm)	315	325	324						15	
Hard. (mg/L)	98								Titr	
Alk. (mg/L)	60								Titr	
TRC (mg/L) 3/18	<0.02								22	
NH_3 (mg/L) 3/18	<1.0								HA#1	
Conc.: 6.25%										
pH	7.4	7.0	7.1							
D.O. (mg/L)	7.0	6.5	6.2							
Temp. (°C)	—									
Cond. (µS/cm)	307	314	312							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 12.5%										
pH	6.9	7.0	6.8							
D.O. (mg/L)	7.0	6.2	6.4							
Temp. (°C)	—									
Cond. (µS/cm)	301	318	314							
Conc.: 25%										
pH	6.3	6.1	6.6							
D.O. (mg/L)	7.1	6.0	5.8							
Temp. (°C)	—									
Cond. (µS/cm)	298	309	316							
Date:	3/25/15	4/1/15	4/3/15							
Time:	1125	1000	0830							
Initials:	DAP	DAP	DAP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DAP 10/6/15 E
② AMR 12/15/15 E

① ALL

CHRONIC CHEMICAL DATA (~~INITIAL~~)

4 ①

QA: AR 10/9/15

Project Number: 15001-268-006

Test Species: ~~Rana~~ Lithobates pipiens ①

%	Day 0 (new)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: 50.0%									All Conc.	
pH	4.3	5.4	5.6							
D.O. (mg/L)	7.0	5.8	5.9							
Temp. (°C)	–									
Cond. (µS/cm)	314	338	344							
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 100%										
pH	3.7									
D.O. (mg/L)	7.2									
Temp. (°C)	20.5									
Cond. (µS/cm)	405									
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	3/25/15	4/1/15	4/3/15							
Time:	1125	1000	0930							
Initials:	DAP	DAP	DAP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DAP 10/6/15 E
② Am 12/15/15 E

③ 4 **DAILY TOXICITY TEST LOG** QA: AR 10/9/15

Project Number:	15001-268-006
Test Species:	② ~~Rana pipiens~~ Lithobates

General Comments	Random Chart: FHM Random Bd, Not Rewarded Min/Max Thermometer # M-15	Feeding Grnd Tetramin/ ~1 pinch per rep	Initials/Date
Test Day 0	Test Solution Mixed at: 1100-1145 Test Organisms Added at: 1200	Fed @	DAP 3/25/15
Test Day 1	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DAP 3/26/15
Test Day 2	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @ 1100 ① ~1 pinch of Tetrafin food added per rep	SP 3/27/15
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @	DAP 3/28/15
Test Day 4	Real Time: 24 °C Min-Max Range: 23-26 °C	Fed @	DAP 3/29/15 ②
Test Day 5	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @ 1000 ① ~1 pinch of tetrafin added / rep	SP 3/30/15
Test Day 6	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @	DAP 3/31/15
Test Day 7	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @ 1055 ~1 pinch of tetrafin added / rep	SP 4/1/15
Test Day 8	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @	DAP 4/2/15

① Began feeding on day ② ~~0~~ 2; Gosner Stage ~ 25-27. DAP ③ DAP 3/31/15 E

③ DAP 10/5/15 E

① 4

DAILY TOXICITY TEST LOG

QA: AR 10/9/15

Project Number:	15001-268-006
Test Species:	① ~~Rana~~ pipiens Lithobates

General Comments	Random Chart: not recorded	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 24 °C	Min-Max Range: 24-24 °C	Fed @ 0930 ~1 pinch of Tetrafin/rep	SP 4/3/15
Test Day 10	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 11	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 12	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 13	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 14	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 15	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 16	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 17	Real Time: °C	Min-Max Range: °C	Fed @	

① DSP 10/6/15 E

CETIS Analytical Report

Lithobates pipiens (1)

~~Fathead Minnow 96-h~~ Acute Survival Test

Report Date:	12 Oct-15 16:57 (p 1 of 2)
Test Code:	468-006 | 14-5502-6152
	TRE Environmental Strategies, LLC

Analysis ID:	03-3171-4901	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Oct-15 16:57	**Analysis:**	Binomial Method	**Official Results:**	Yes

Batch ID:	19-4477-9963	**Test Type:**	Survival ~~(96h)~~ (1)	**Analyst:**	Lab Tech
Start Date:	25 Mar-15 ~~14:50~~ 1200 (1)	**Protocol:**	~~EPA/821/R-02-012 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water (1)
Ending Date:	~~29 Mar-15 13:55~~	**Species:**	~~Pimephales promelas~~ Lithobates pipiens	**Brine:**	
Duration:	~~95h~~ (1)	**Source:**	~~In House Culture~~ Caroline Biological (1)	**Age:**	

Sample ID:	07-8220-9724	**Code:**	2E9F92BC	**Client:**	Confidential
Sample Date:	~~24 Mar-15~~ (1)	**Material:**	~~Not Applicable~~ NTO (1)	**Project:**	Special Studies
Receive Date:		**Source:**	~~Reference Toxicant~~ (1)		
Sample Age:	~~39h~~ (1)	**Station:**			

Binomial/Graphical Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.433	0	270.8	191.5	383

48h Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**A**	**B**
0	Dilution Water	2	1	1	1	0	0	0.0%	0.0%	10	10
24.5		2	1	1	1	0	0	0.0%	0.0%	10	10
47.9		2	1	1	1	0	0	0.0%	0.0%	10	10
95.8		2	1	1	1	0	0	0.0%	0.0%	10	10
191.5		2	1	1	1	0	0	0.0%	0.0%	10	10
383		2	0	0	0	0	0		100.0%	0	10

48h Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	1	1
24.5		1	1
47.9		1	1
95.8		1	1
191.5		1	1
383		0	0

48h Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	5/5	5/5
24.5		5/5	5/5
47.9		5/5	5/5
95.8		5/5	5/5
191.5		5/5	5/5
383		0/5	0/5

(1) DAP 10/12/15 E

CETIS Analytical Report

Lithobates pipiens ①

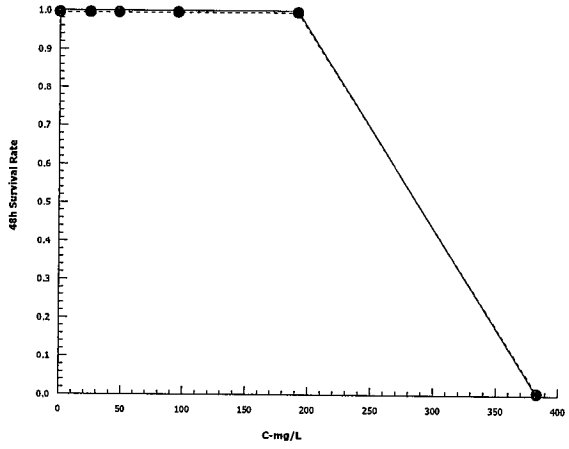
~~Fathead Minnow 96-h~~ Acute Survival Test

Report Date:	12 Oct-15 16:57 (p 2 of 2)
Test Code:	468-006 | 14-5502-6152

TRE Environmental Strategies, LLC

Analysis ID:	03-3171-4901	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Oct-15 16:57	**Analysis:**	Binomial Method	**Official Results:**	Yes

Graphics



① 10/12/15 E
DAP

CETIS Analytical Report

Lithobates pipiens (1)

Report Date:	12 Oct-15 16:57 (p 1 of 2)
Test Code:	468-006 | 14-5502-6152

~~Fathead Minnow 96-h~~ Acute Survival Test 7 day (1)

TRE Environmental Strategies, LLC

Analysis ID:	01-8858-5255	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Oct-15 16:57	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Batch ID:	19-4477-9963	**Test Type:**	Survival ~~(96h)~~ (1)	**Analyst:**	Lab Tech
Start Date:	25 Mar-15 ~~14:50~~ 1200 (1)	**Protocol:**	~~EPA/821/R-02-012 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	~~29 Mar-15 13:55~~ (1)	**Species:**	~~Pimephales promelas~~ Lithobates pipiens	**Brine:**	
Duration:	~~95h~~ (1)	**Source:**	~~In-House Culture~~ Carolina Biological (1)	**Age:**	

Sample ID:	07-8220-9724	**Code:**	2E9F92BC	**Client:**	Confidential
Sample Date:	~~24 Mar-15~~ (1)	**Material:**	~~Not Applicable~~ NTO (1)	**Project:**	Special Studies
Receive Date:		**Source:**	~~Reference Toxicant~~ (1)		
Sample Age:	~~39h~~ (1)	**Station:**			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.403	0.02943	252.8	220.7	289.5

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

(1) ~~96h~~ 7-Day Survival Rate Summary

C-mg/L	Control Type	Count	Calculated Variate(A/B) Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	2	1	1	1	0	0	0.0%	0.0%	10	10
24.5		2	1	1	1	0	0	0.0%	0.0%	10	10
47.9		2	0.9	0.8	1	0.1	0.1414	15.7%	10.0%	9	10
95.8		2	1	1	1	0	0	0.0%	0.0%	10	10
191.5		2	1	1	1	0	0	0.0%	0.0%	10	10
383		2	0	0	0	0	0		100.0%	0	10

(1) ~~96h~~ 7-Day Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	1	1
24.5		1	1
47.9		0.8	1
95.8		1	1
191.5		1	1
383		0	0

(1) ~~96h~~ 7-Day Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	5/5	5/5
24.5		5/5	5/5
47.9		4/5	5/5
95.8		5/5	5/5
191.5		5/5	5/5
383		0/5	0/5

(1) DAP 10/12/15 E

CETIS Analytical Report

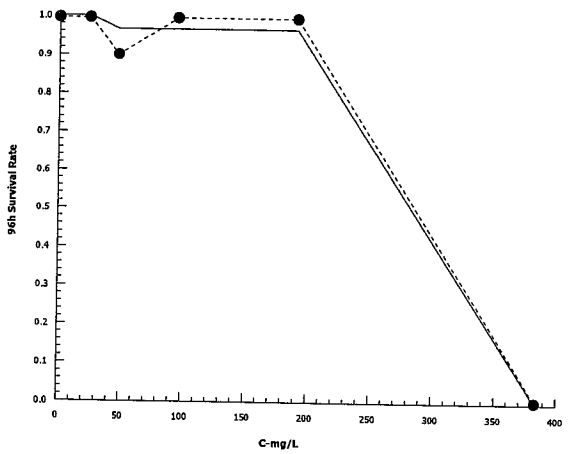
Lithobates pipiens ①

~~Fathead Minnow 96-h~~ Acute Survival Test 7-Day ①

Report Date:	12 Oct-15 16:57 (p 2 of 2)
Test Code:	468-006 | 14-5502-6152
TRE Environmental Strategies, LLC	

Analysis ID:	01-8858-5255	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Oct-15 16:57	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



① DSP 10/12/15 E

Test Failed due to control failure

QA Form No. 051
Revision 5
Effective 02/14

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Acute

Project Number: 15001-268-007 (1)

Test Substance: NTO - Unadjusted (ambient pH)

Species: Lithobates (1) ~~Rana~~ pipiens

Dilution Water: Mod Hard

Organism Lot or Batch Number: 15-008

Concurrent Control Water: NA

Age: ~18d old (Stage 25+)

Supplier: Carolina Biological

Date and Time Test Began: 3/25/15 @ 1200

Date and Time Test Ended: 4/3/15 @ 0855

Protocol Number:

Investigator(s): DAP/SP/MJ/MW

Background Information

Type of Test: Static ~~Renewal~~ (1)

pH control?: Yes No

If yes, give % CO_2: NA

Test Temperature: 25 ± 1 °C

Env. Chmbr/Bath #: 1

Test Chmbrs: 500-ml cups ~~/beakers~~ (2)

Photoperiod: 16 h light : 8 h dark

Light intensity: 50-100 ft-c.

Test Solution Vol.: 250 ml

Replicates per Treatment: 2

Length of Test: (1) ~~14~~ 7-21 days

Organisms per Replicate: 5

Type of Food and Quantity per Chamber: Grnd Tetramin; ~ 0.2 g

Feeding Frequency: ~ every ~~2~~ 3 d (1) after Gosner stage 25

Test Substance Characterization Parameters and Frequency:

Hardness: Alkalinity: NH_3: TRC:

pH: Init and Term Conductivity: Init and Term

Test Concentrations (Volume:Volume): MH, 0.31, 0.625, 1.25, 2.5, and 5% NTO Saturated Stock
(5% is considered 100% for remainder of data pacakge)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: _____ to _____ IC_{25}: _____

Hist. 95% Control Limits: _____ to _____ Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Study Director Initials: DAP Date: 3/25/15

(1) DAP 10/26/15 E

(2) DAP 10/26/15 - Plastic cups were used

TEST SUBSTANCE USAGE LOG

Project Number: 15001-268-007 (4 ①)

d/A: 10/30/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: @ NA	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (Grab or Comp)	NA			
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	11553			
Concurrent Control Water RW#	NA			
Date(s) Used	3/25/15			

Preparation of Test Solutions

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	500	500	0	1000	1000			
6.25%	31.25	468.75	500	63	938	1000			
12.5%	62.5	437.5	500	125	875	1000			
25%	125	375	500	250	750	1000			
50%	250	250	500	500	500	1000			
100%	500	0	500						
Total	968.75	2031.25	3000	938	4063	5000			

Initials / Date		3/25/15 DSP	
Initials / Date			
Initials / Date		Note: 1000 ml prepared	
Initials / Date		for tests 006 and	
Initials / Date		007 · DSP 3/25/15	
Initials / Date			
Initials / Date			
Initials / Date			

① DSP 10/26/15 E

③ Northern Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

QA: MR 10/30/15

Project Number: 15001-~~2~~4③68-007

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
MH	A	5	5	5	4	4	4	4	4	
NTU=	B	5	5	5	3	3	3	3	3	
0	C	5	①							
	D	5								
6.25%	A	5	5	5	5	5	5	5	5	
NTU=	B	5	5	5	5	5	5	5	5	
24.5	C	5								
	D	5								
12.5%	A	5	5	5	4	4	4	4	4	
NTU=	B	5	5	5	5	5	5	5	5	
47.9	C	5								
	D	5								
25%	A	5	5	5	5	5	5	5	5	
NTU=	B	5	5	5	5	5	5	5	4	
95.8	C	5								
	D	5								
50.0%	A	5	5	4	4	4	4	4	4	
NTU=	B	5	5	4	4	4	4	4	4	
191.5	C	5								
	D	5								
100.0%	A	5	80	0	–	–	–	–	–	
NTU=	B	5	② 80	0	–	–	–	–	–	
383	C	5								
	D	5								
	A									
	B									
	C									
	D									
	Date:	3/25/15	3/26/15	3/27/15	3/28/15	3/29/15	3/30/15	3/31/15	4/1/15	
	Time:	1200	1710	0825	1255	1335	0920	1430	0920	
	Initials:	DAP	DAP	SP	MW	MW	SP	DAP	SP	

① DAP 3/25/15 E – only 2 reps ② DAP 3/26/15 E ③ DAP 10/26/15 E

① Northern Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

QA: AR210/30115

Project Number: 15001-268-007 ④ ①

%Conc.	Test Replicate	Number of Surviving Organisms Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Remarks
MH	A	4	4							
	B	3	3							
	C									
	D									
6.25%	A	5	5							
	B	5	5							
	C									
	D									
12.5%	A	4	4							
	B	5	5							
	C									
	D									
25%	A	5	5							
	B	4	4							
	C									
	D									
50.0%	A	4	4							
	B	4	4 [illegible]							Δ: H2O cloudy + mercky.
	C									
	D									
100.0%	A									
	B									
	C									
	D									
	A									
	B									
	C									
	D									
	Date:	4/2/15	4/3/15							
	Time:	1650	0855							
	Initials:	DSP	SP							

① DSP 10/26/15 E
④ BMM 12/15/15 E

CHRONIC CHEMICAL DATA (~~INITIAL~~ ALL)

Project Number: 15001-~~2~~4 68-007

Test Species: ~~Rana pipiens~~ Lithobates

%	Day 0 (New)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: MH									All Conc.	
pH	8.2	8.4	8.4						F423	
D.O. (mg/L)	7.2	7.0	6.6						5	
Temp. (°C)	19.1								L-5	
Cond. (µS/cm)	315	325	324						15	
Hard. (mg/L)	98								Titr	
Alk. (mg/L)	60								Titr	
TRC (mg/L) 3/18	<0.02								22	
NH_3 (mg/L) 3/18	<1.0								HA1	
Conc.: 6.25%										
pH	7.4	7.0	7.1							
D.O. (mg/L)	7.0	6.4	6.1							
Temp. (°C)	—									
Cond. (µS/cm)	307	318	315							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 12.5%										
pH	~~7.0~~ 6.9	6.8	6.6							
D.O. (mg/L)	7.0	6.5	6.2							
Temp. (°C)	—									
Cond. (µS/cm)	301	312	324							
Conc.: 25%										
pH	6.3	6.2	6.2							
D.O. (mg/L)	7.1	7.0	6.0							
Temp. (°C)	—									
Cond. (µS/cm)	298	306	312							
Date:	3/25/15	4/1/15	4/3/15							
Time:	1125	1010	0945							
Initials:	DAP	DAP	DAP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DAP 3/25/15 E ② DAP 10/26/15 E ③ am 12/15/15 E

① ALL

CHRONIC CHEMICAL DATA (~~INITIAL~~)

4 ①

Project Number: 15001-268-007

Test Species: ~~Rana~~ Lithobates pipiens ①

%	Day 0 (new)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: 50.0%									All Conc.	
pH	4.3	4.8	5.3							
D.O. (mg/L)	7.0	5.6	5.4							
Temp. (°C)	—									
Cond. (µS/cm)	314	322	340							
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 100%										
pH	3.7									
D.O. (mg/L)	7.2									
Temp. (°C)	20.5									
Cond. (µS/cm)	405									
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	3/25/15	4/1/15	4/3/15							
Time:	1125	1010	0945							
Initials:	DAP	DAP	DAP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DAP 10/26/15 E
② Dmm 12/15/15 E

4 (3) **DAILY TOXICITY TEST LOG**

Project Number:	15001-268-007
Test Species:	~~Rana pipiens~~

(3) Lithobates

General Comments	Random Chart: Not Recorded FHM Random Bd, Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 0	Test Solution Mixed at: 1100-1145 Test Organisms Added at: 1200	Fed @	DAP 3/25/15
Test Day 1	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DAP 3/26/15
Test Day 2	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @ ~~1100~~ (1) ~1 pinch of Tetrafin food added per rep	~~SP 3/27/15~~ (1)
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C (2)	Fed @ 1100 ~1 pinch of tetramin food added/rep	SP 3/27/15 DAP 3/27/15
Test Day 4	Real Time: 24 °C Min-Max Range: 23-26 °C	Fed @	DAP 3/28/15
Test Day 5	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @ 1000 ~1 pinch of tetrafin added/rep	SP 3/30/15
Test Day 6	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @	DAP 3/31/15
Test Day 7	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @ 1055 ~1 pinch of tetrafin added /rep	SP 4/1/15
Test Day 8	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DAP 4/2/15

(1) SP 3/27/15 - WP

(2) SP 3/30/15 - C, 1st day off feeding was day 2, not day 3 on 3/27/15. data was originally written in right place (day 2). disregard day 3 feeding data

(3) DAP 10/26/15 E

4 ①

DAILY TOXICITY TEST LOG

Project Number:	15001-268-007
Test Species:	① ~~Rana~~ pipiens Lithobates

General Comments	Random Chart: not recorded Min/Max Thermometer # M-15		Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 24 °C	Min-Max Range: 24-24 °C	Fed @ 0930 ~1pinch of Tetrafin/rep	SO 4/3/15
Test Day 10	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 11	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 12	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 13	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 14	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 15	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 16	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 17	Real Time: °C	Min-Max Range: °C	Fed @	

① DSP 10/26/15 E

CETIS Analytical Report

Lithobates pipiens ①

~~Fathead Minnow 96-h~~ Acute Survival Test

Report Date:	09 Dec-15 12:21 (p 1 of 2)
Test Code:	468-007 | 08-3105-5152

TRE Environmental Strategies, LLC

Analysis ID:	12-9840-7812	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Dec-15 12:21	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	19-4477-9963	**Test Type:**	Survival ~~(96h)~~ ①	**Analyst:**	Lab Tech
Start Date:	25 Mar-15 12:00	**Protocol:**	~~EPA/821/R-02-012 (2002)~~ ①	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	03 Apr-15 08:55	**Species:**	~~Pimephales promelas~~ Lithobates pipiens	**Brine:**	
Duration:	8d 21h	**Source:**	~~In-House Culture~~ Carolina Biological	**Age:**	~ 18 d ①
Sample ID:	14-1246-9521	**Code:**	54309311	**Client:**	Confidential
Sample Date:	~~26 Oct-15 13:39~~ ①	**Material:**	~~Not Applicable~~ NSO ①	**Project:**	Special Studies
Receive Date:	~~26 Oct-15 13:39~~ ①	**Source:**	Research		
Sample Age:	NA	**Station:**			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.373	0.03806	235.8	197.9	280.9

48h Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**A**	**B**
0	Dilution Water	2	1	1	1	0	0	0.0%	0.0%	10	10
24.5		2	1	1	1	0	0	0.0%	0.0%	10	10
47.9		2	1	1	1	0	0	0.0%	0.0%	10	10
95.8		2	1	1	1	0	0	0.0%	0.0%	10	10
191.5		2	0.8	0.8	0.8	0	0	0.0%	20.0%	8	10
383		2	0	0	0	0	0		100.0%	0	10

48h Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	1	1
24.5		1	1
47.9		1	1
95.8		1	1
191.5		0.8	0.8
383		0	0

48h Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	5/5	5/5
24.5		5/5	5/5
47.9		5/5	5/5
95.8		5/5	5/5
191.5		4/5	4/5
383		0/5	0/5

① DSP 12/9/15 E

CETIS Analytical Report

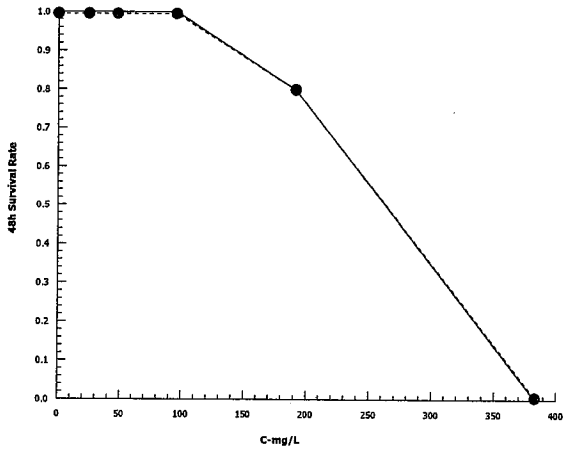
Lithobates pipiens (1)

Report Date:	09 Dec-15 12:21 (p 2 of 2)		
Test Code:	468-007 | 08-3105-5152		

~~Fathead Minnow 96-h~~ Acute Survival Test — **TRE Environmental Strategies, LLC**

Analysis ID:	12-9840-7812	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	09 Dec-15 12:21	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



(1) DSP 12/9/15 E

CETIS Analytical Report

Lithobates pipiens

~~Fathead Minnow 96-h~~ Acute Survival Test 7-Day (1)

Report Date:	26 Oct-15 13:44 (p 1 of 2)
Test Code:	468-007 | 08-3105-5152

TRE Environmental Strategies, LLC

Analysis ID:	03-4985-1184	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Oct-15 13:44	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Batch ID:	19-4477-9963	**Test Type:**	Survival ~~(96h)~~ (1)	**Analyst:**	Lab Tech
Start Date:	25 Mar-15 12:00	**Protocol:**	~~EPA/821/R-02-012 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	03 Apr-15 08:55	**Species:**	~~Pimephales promelas~~ Lithobates pipiens	**Brine:**	
Duration:	8d 21h	**Source:**	~~In-House Culture~~ Carolina Biological (1)	**Age:**	~ 18 days

Sample ID:	14-1246-9521	**Code:**	54309311	**Client:**	Confidential
Sample Date:	~~26 Oct-15 13:39~~ (1)	**Material:**	~~Not Applicable~~ NTO (1)	**Project:**	Special Studies
Receive Date:	~~26 Oct-15 13:39~~ (1)	**Source:**	Research		
Sample Age:	NA	**Station:**			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0.3	0.00%	2.407	0.02664	255.2	225.7	288.5

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.7	0.9 - NL	Yes	Below Acceptability Criteria

~~96h~~ 7-Day (1) Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	2	0.7	0.6	0.8	0.1	0.1414	20.2%	0.0%	7	10
24.5		2	1	1	1	0	0	0.0%	-42.9%	10	10
47.9		2	0.9	0.8	1	0.1	0.1414	15.7%	-28.6%	9	10
95.8		2	0.9	0.8	1	0.1	0.1414	15.7%	-28.6%	9	10
191.5		2	0.8	0.8	0.8	0	0	0.0%	-14.3%	8	10
383		2	0	0	0	0	0		100.0%	0	10

~~96h~~ 7-Day (1) Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	0.8	0.6
24.5		1	1
47.9		0.8	1
95.8		1	0.8
191.5		0.8	0.8
383		0	0

~~96h~~ 7-Day (1) Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	4/5	3/5
24.5		5/5	5/5
47.9		4/5	5/5
95.8		5/5	4/5
191.5		4/5	4/5
383		0/5	0/5

(1) DAP 10/26/15 E

CETIS Analytical Report

Lithobates pipiens ①

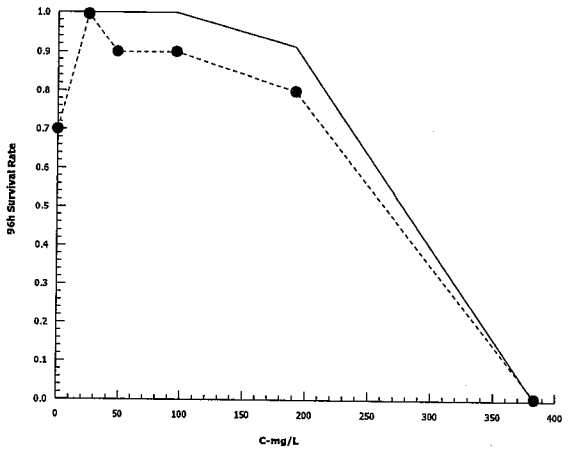
Report Date:	26 Oct-15 13:44 (p 2 of 2)		
Test Code:	468-007 | 08-3105-5152		

~~Fathead Minnow 96-h~~ Acute Survival Test 7-Day ①

TRE Environmental Strategies, LLC

Analysis ID:	03-4985-1184	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Oct-15 13:44	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Graphics



① DEP 10/26/15 E

QA: AR 10/16/15

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Acute

Project Number: 15001-268-008 4 ①

Test Substance: NTO - pH-adjusted

Species: ~~Rana pipiens~~ Lithobates ①

Dilution Water: Mod Hard

Organism Lot or Batch Number: 15-012

Concurrent Control Water: NA

Age: 72 h (24-72 h) ~ 48-

Supplier: Carolina Biological

Date and Time Test Began: 3/25/15 @ 1700

Date and Time Test Ended: 4/7/15 @ 0900

Protocol Number:

Investigator(s): DAP / SP / MW

Background Information

Type of Test: Static ~~Renewal~~ ①

pH control?: Yes **No**

If yes, give % CO_2: NA

Test Temperature: 25 ± 1 °C

Env. Chmbr/Bath #: 1

Test Chmbrs: 500-ml cups ~~/beakers~~ ②

Photoperiod: 16 h light : 8 h dark

Light intensity: 50-100 ft-c.

Test Solution Vol.: 250 ml

Replicates per Treatment: 2

Length of Test: ① ~~14~~ 7-21 days

Organisms per Replicate: 5

Type of Food and Quantity per Chamber: Grnd Tetramin; ~ 0.2 g

Feeding Frequency: ~ every 2 d after Gosner stage 25

Test Substance Characterization Parameters and Frequency:

Hardness: Alkalinity: NH_3: TRC:

pH: Init and Term Conductivity: Init and Term

Test Concentrations (Volume:Volume): MH, 12.5, 25, and 50% NTO Saturated Stock
(50% is considered 100% for remainder of data pacakge)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: _____ to _____ IC_{25}: _____

Hist. 95% Control Limits: _____ to _____ Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Tadpoles @ Gosner stage 24 @ Setup. Gills still apparent on left side

Study Director Initials: DAP Date: 3/25/15

① DAP 10/14/15 E ② DAP 10/14/15 – Plastic cups were used

TEST SUBSTANCE USAGE LOG

Project Number: 15001-268-008 4 (2)

QA: AR 10/16/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: @ NA	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (Grab or Comp)	NA			
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	1155 3			
Concurrent Control Water RW#	NA			
Date(s) Used	3/25/15			

Preparation of Test Solutions

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	500	500	0	1000	1000			
	0	500	500	0	1000	1000			
	0	500	500	0	1000	1000			
25%	125	375	500	250	750	1000			
50%	250	250	500	500	500	1000			
100%	500	0	500	1000	0	1000			
Total	875	2125	3000	1750	4250	6000			

Initials / Date	~~3/25/15 DAP~~ (1)	3/25/15 DAP	
Initials / Date			
Initials / Date		Note: 1000 ml prepared	
Initials / Date		for tests 008 + 009	
Initials / Date		DAP 3/25/15	
Initials / Date			
Initials / Date			
Initials / Date			

(1) DAP 3/25/15 E (2) DAP 10/13/15 E

QA Form No. 060
Revision 3
Effective 02/14

③ Northern Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-~~2~~4-008 ③ QA: AR 10/16/15

%Conc.	Test Replicate	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
		Number of Surviving Organisms								
MH	A	5	5	5	5	5	5	5	5	
NTO =	B	5	5	4	4	4	4	4	4	
0	C									
	D									
0.00%	A									
	B									
	C									
	D									
0.0%	A									
	B									
	C									
	D									
25%	A	5	5	~~4~~ ① 5	5	5	5	5	5	
NTO	B	5	5	5	5	5	5	5	4	
= 943	C									
	D									
50.0%	A	5	5	5	5	5	5	5	5	
NTO	B	5	5	~~4~~ ① 5	4^	4	4	4	4	D1 NF
= 1840	C									
	D									
100.0%	A	5	5	~~4~~ ① 5	4^	4	4	4	4^A2	D1 NF, D2 food from day 5 still present.
NTO =	B	5	5	~~5~~ ② 5	5	5	5	5	5^A2	
3710	C									
	D									
	A									
	B			→	48 h	LC50 > 3710			→	7 d LC50 > 3710
	C					mg/L				mg/L
	D									
Date:		3/25/15	3/26/15	3/27/15	3/28/15	3/29/15	3/30/15	3/31/15	4/1/15	
Time:		1700	1720	0835	1300	1310	0925	1430	0930	
Initials:		DSP	DSP	SP	AB	MW	SP	DSP	SP	

① SP 3/27/15 - E, Still have all 5 alive ③ DSP 10/13/15 E
② SP 3/27/15 - E,

(2) Northern Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

QA: AR 10/16/15

Project Number: 15001-~~2~~4-008 (2)

%Conc.	Test Replicate	Number of Surviving Organisms								
		Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Remarks
MH	A	5	5							
	B	4	4							
	C									
	D									
0.00%	A									
	B									
	C									
	D									
0.0%	A									
	B									
	C									
	D									
25%	A	5	5							
	B	4	4							
	C									
	D									
50.0%	A	5	5							
	B	4	4							
	C									
	D									
100.0%	A	4	4							
	B	5	5							
	C									
	D									
	A									
	B									
	C									
	D									
Date:		4/2/15	4/3/15							
Time:		(1) 1645	0900							
Initials:		DAP	SP							

(1) DAP 4/2/15 E (2) DAP 10/13/15 E (3) DMM 12/15/15 E

① ALL

4 ① **CHRONIC CHEMICAL DATA** ~~(INITIAL)~~

QA: AR 10/16/15

Project Number: 15001-268-008

Test Species: ~~*Rana pipiens*~~ Lithobates ①

%	Day 0 (New)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: MH									All Conc.	
pH	8.1	8.3	8.3							
D.O. (mg/L)	7.2	6.2	6.0							
Temp. (°C)	21.3									
Cond. (µS/cm)	308	322	326							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 0.00%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 0.0%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 25%										
pH	7.9	8.3	8.2							
D.O. (mg/L)	7.1	7.0	6.2							
Temp. (°C)										
Cond. (µS/cm)	848	864	872							
Date:	3/25/15	4/1/15	4/3/15							
Time:	1715	1020	1030							
Initials:	DAP	DAP	DAP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DAP 10/14/15 E ② DAP 12/15/15 E

QA: AR 10/16/15

CHRONIC CHEMICAL DATA ~~(INITIAL)~~ ALL

Project Number: 15001-268-008

Test Species: ~~Rana~~ Lithobates pipiens

%	Day 0 (new)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: 50.0%									All Conc.	
pH	7.7	7.5	7.6							
D.O. (mg/L)	7.0	6.6	6.2							
Temp. (°C)										
Cond. (µS/cm)	1430	1440	1442							
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 100%										
pH	7.4	7.2	7.3							
D.O. (mg/L)	7.0	6.2	6.0							
Temp. (°C)	22.2									
Cond. (µS/cm)	2520	2535	2542							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	3/25/15	4/1/15	4/3/15							
Time:	1715	1020	1030							
Initials:	DMP	DMP	DMP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DMP 10/14/15 E ② AM 12/15/15 E

4 ② ①

DAILY TOXICITY TEST LOG

QA: AR 10/16/15

Project Number:	15001-268-008
Test Species:	~~Rana~~ *pipiens*

① Lithobates

General Comments	Random Chart: FHM Random Bd Not Recorded Min/Max Thermometer # M-15	Feeding Grnd Tetramin ~1 pinch per rep	Initials/Date
Test Day 0	Test Solution Mixed at: 1300-1430 Test Organisms Added at: 1700	Fed @	DAP 3/25/15
Test Day 1	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DAP 3/26/15
Test Day 2	Real Time: ~~25~~ 24 ② °C Min-Max Range: 23-24 °C	Fed @ 1100 ~1 pinch of tetrafin food added / rep	DAP for SP 3/27/15
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @	DAP 3/28/15
Test Day 4	Real Time: 24 °C Min-Max Range: 23-26 °C	Fed @	DAP 3/29/15
Test Day 5	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @ 1000 ~1 pinch of tetrafin added / rep	SP 3/30/15
Test Day 6	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @	DAP 3/31/15
Test Day 7	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @ 1055 ~1 pinch of tetrafin added / rep	SP 4/1/15
Test Day 8	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @	DAP 4/2/15

① DAP 10/14/15 E ② DAP 10/14/15 E- Late Entry

4 ①

DAILY TOXICITY TEST LOG

QA: AR 10/16/15

Project Number:	15001-268-008
Test Species:	~~Rana pipiens~~

① Lithobates

General Comments	Random Chart: Not Rec.	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 24 °C	Min-Max Range: 24-24 °C	Fed @ 0930 ~1 pinch tetrafin/rep	SP 4/3/15
Test Day 10	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 11	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 12	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 13	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 14	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 15	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 16	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 17	Real Time: °C	Min-Max Range: °C	Fed @	

① DSP 10/14/15

QA: MR 10/16/15

TOXICITY DATA PACKAGE COVER SHEET

Test Type:	Acute	Project Number:	15001~~2~~68-009 (4) (1)
Test Substance:	NTO - pH-adjusted	Species:	Lithobates ~~Rana~~ pipiens (1)
Dilution Water:	Mod Hard	Organism Lot or Batch Number:	15-~~012~~ 008 (1)
Concurrent Control Water:	NA	Age:	~18 d old (Stage 25+) Supplier: Carolina Biological
Date and Time Test Began:	3/25/15 @ 1700	Date and Time Test Ended:	4/3/15 @ 0905
Protocol Number:		Investigator(s):	DAP/SP/MW/AY

Background Information

Type of Test:	Static ~~Renewal~~ (1)	pH control?:	Yes **No**
		If yes, give % CO_2:	NA
Test Temperature:	25 ± 1 °C	**Env. Chmbr**/Bath #:	1
		Test Chmbrs:	500-ml cups/~~beakers~~ (2)
Photoperiod:	16 h light : 8 h dark	Light intensity:	50-100 ft-c.
Test Solution Vol.:	250 ml	Replicates per Treatment:	2
Length of Test:	(1) 7 ~~14~~-21 days	Organisms per Replicate:	5
Type of Food and Quantity per Chamber:	Grnd Tetramin; ~ 0.2 g	Feeding Frequency:	~ every 2 d after Gosner Stage 25

Test Substance Characterization Parameters and Frequency:

Hardness: Alkalinity: NH_3: TRC:

pH: Init and Term Conductivity: Init and Term

Test Concentrations (Volume:Volume): MH, 12.5, 25, and 50% NTO Saturated Stock
(50% is considered 100% for remainder of data pacakge)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: _____ to _____ IC_{25}: _____

Hist. 95% Control Limits: _____ to _____ Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Approx. Gosner Stage 25 - No limb buds present

Study Director Initials: DAP Date: 3/25/15

(1) DAP 10/13/15 E (2) DAP 10/13/15 - Plastic cups were used

TEST SUBSTANCE USAGE LOG

Project Number: 15001-~~2~~68-009 (1 4)

QA: AR 10/16/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: @ NA	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (Grab or Comp)	NA			
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	11553			
Concurrent Control Water RW#	NA			
Date(s) Used	3/25/15			

Preparation of Test Solutions

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	500	500	0	1000	1000			
	0	500	500	0	1000	1000			
	0	500	500	0	1000	1000			
25%	125	375	500	250	750	1000			
50%	250	250	500	500	500	1000			
100%	500	0	500	1000	0	1000			
Total	875	2125	3000	1750	4250	6000			

Initials / Date		3/25/15 DAP	
Initials / Date			
Initials / Date		Note: 1000 ml prepared	
Initials / Date		for tests 008 + 009	
Initials / Date		DAP 3/25/15	
Initials / Date			
Initials / Date			
Initials / Date			

DAP 10/13/15 E

QA Form No. 060
Revision 3
Effective 02/14

(1) Northern Leopard Frog (Lithobates Diplens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

(1)

Project Number: 15001-268-009 (4) QA: AR 10/16/15

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
MH	A	5	5	5	5	5	5	5	5	
	B	5	5	5	4	4	4	4	4	
	C									
	D									
0.00%	A									
	B									
	C									
	D									
0.0%	A									
	B									
	C									
	D									
25%	A	5	5	5	4	4	4	4	4	
N TO =	B	5	5	5	4	4	4	4	4	
943	C									
	D									
50.0%	A	5	5	5	4	4	4	4	4	
N TO =	B	5	5	5	4	4	4	4	4	
1840	C									
	D									
100.0%	A	5	5	5	5	5	5	5	5	
N TO =	B	5	5	5	5	5	5	5	5	
3710	C									
	D									
	A									
	B			↳ 48 h LC_{50} > 3710 mg/L					↳ 7d LC_{50} > 3710 mg/L	
	C									
	D									
Date:		3/25/15	3/26/15	3/27/15	3/28/15	3/29/15	3/30/15	3/31/15	4/1/15	
Time:		1700	1720	0845	1300	1305	0930	1430	0925	
Initials:		DAP	DAP	SP	AS	MW	SP	DAP	DAP for SP	

(1) DAP 10/13/15 E

② Northern Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

QA: AR 10/16/15

Project Number: 15001~~2~~4 ② 68-009

%Conc.	Test Replicate	Number of Surviving Organisms Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Remarks
MH	A	① ~~4~~ 5	5							
	B	4	4							
	C									
	D									
0.00%	A									
	B									
	C									
	D									
0.0%	A									
	B									
	C									
	D									
25%	A	4	4							
	B	4	4							
	C									
	D									
50.0%	A	4	4							
	B	4	4							
	C									
	D									
100.0%	A	5	5 Δ1							Δ1 H2O cloudy
	B	5	5 Δ1							
	C									
	D									
	A									
	B									
	C									
	D									
Date:		4/2/15	4/3/15							
Time:		1645	0905							
Initials:		DAP	SP							

① DAP 4/2/15 CF ② DAP 10/13/15 5 ③ Am 12/15/15 E

① ALL

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

4 ①

Project Number: 15001-~~2~~68-009

QA: MC 10/16/15

Test Species: ~~Rana~~ Lithobates ① pipiens

%	Day 0 (New)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: MH									All Conc.	
pH	8.1	8.2	8.4						FM23	
D.O. (mg/L)	7.2	6.4	5.4						5	
Temp. (°C)	21.3								L-5	
Cond. (µS/cm)	308	319	322						15	
Hard. (mg/L)	98								Titr	
Alk. (mg/L)	60								Titr	
TRC (mg/L) 3/19	<0.02								22	
NH_3 (mg/L) 3/18	<1.0								HA 1	
Conc.: 0.00%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 0.0%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 25%										
pH	7.9	8.1	8.2							
D.O. (mg/L)	7.1	6.4	6.0							
Temp. (°C)	—									
Cond. (µS/cm)	848	858	862							
Date:	3/25/15	4/1/15	4/3/15							
Time:	1715	1010	1015							
Initials:	DAP	DSP	DSP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DSP 10/13/15 E ① AM 12/15/15 E

①

① ALL

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

4 ①

Project Number: 15001-268-009

QA: DZ 10/16/15

Test Species: ~~Rana~~ Lithobates pipiens ①

%	Day 0 (new)	Day 7 (old)	Day 9 (old)	Day	Day	Day	Day	Day	Meter #	Remarks
Conc.: 50.0%									All Conc.	
pH	7.7	7.6	7.7							
D.O. (mg/L)	7.0	7.0	6.4							
Temp. (°C)	—									
Cond. (µS/cm)	1430	1432	1455							
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 100%										
pH	7.4	7.3	7.3							
D.O. (mg/L)	7.0	6.4	6.6							
Temp. (°C)	22.2									
Cond. (µS/cm)	2520	2540	2552							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	3/25/15	4/1/15	4/3/15							
Time:	1715	1010	1015							
Initials:	DAP	DAP	DAP							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25°C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25°C.

① DAP 10/13/15 E; ② BM 12/15/15 E

4 (2) **DAILY TOXICITY TEST LOG** QA: MR 10/10/15

Project Number:	15001-268-009
Test Species:	~~Rana~~ pipiens

(2) Lithobates

General Comments	FHM Random Bd Random Chart: Not Recorded Min/Max Thermometer # M-15	Feeding Grnd Tetramin / ~1 pinch per rep	Initials/Date
Test Day 0	Test Solution Mixed at: 1300 - 1430 Test Organisms Added at: 1700	Fed @	DSP 3/25/15
Test Day 1	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DSP 3/26/15
Test Day 2	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @ 1100 ~1 pinch of tetrafin food added/rep	SP 3/27/15
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @ 1100 (1) ~1 pinch of tetramin food added/rep	SP (1) ~~3/27/15~~ DSP 3/28/15
Test Day 4	Real Time: 24 °C Min-Max Range: 23-26 °C	Fed @	DSP 3/29/15
Test Day 5	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @ 1000 ~1 pinch of tetrafin food added/rep	SP 3/30/15
Test Day 6	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @	DSP 3/31/15
Test Day 7	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @ 1055 ~1 pinch of tetrafin added/rep	SP 4/1/15
Test Day 8	Real Time: 24 °C Min-Max Range: 23-25 °C	Fed @	DSP 4/2/15

(1) SP 3/30/15 - E/WP, 1st feeding was on day 2 not day 3 (2) DSP 10/13/15 E

① 4

DAILY TOXICITY TEST LOG

Project Number: 15001268-009
Test Species: ~~Rana~~ *pipiens* ① Lithobates

QA: AR 10/2 16/15 ②

General Comments	Random Chart: Not recorded Min/Max Thermometer # M-15			Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 24 °C	Min-Max Range: 24-24 °C		Fed @ 0930 ~1 pinch Tetrafin/rep	SP 4/3/15
Test Day 10	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 11	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 12	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 13	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 14	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 15	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 16	Real Time: °C	Min-Max Range: °C		Fed @	
Test Day 17	Real Time: °C	Min-Max Range: °C		Fed @	

① ASP 10/13/15 E ② AR 10/16/15E

US Army Public Health Center
Aberdeen Proving Ground, MD

Study Contract Title: One Generation Amphibian Study
W91ZLK-14-P-0372

APPENDIX C

14-DAY AND 28-DAY LARVAL GROWTH DATA PACKAGES & STATISTICAL ANALYSES

Prepared by

TRE Environmental Strategies, LLC
Fort Collins, CO

March 2016

TRE Project Number: 15001-468-710

TOXICITY DATA PACKAGE COVER SHEET

QA: AZ 10/23/15

Test Type:	Chronic	Project Number:	15001-468-010 (Sacf. Reps)
Test Substance:	NTO - pH-adjusted	Species:	*Rana pipiens*
Dilution Water:	Mod Hard	Organism Lot or Batch Number:	15-018
Concurrent Control Water:	NA	Age: ~24 h post hatch (24-72 h)	Supplier: Carolina Biological
Date and Time Test Began:	5/20/15 @ 1330	Date and Time Test Ended:	6/3/15 @ 1050
Protocol Number:		Investigator(s):	DAP / [illegible]

Background Information

Type of Test:	Static-Renewal	pH control?: Yes No If yes, give % CO_2:	NA
Test Temperature:	(2) 22 ± ~~2~~ 3 °C	Env. Chmbr/Bath #: 1	Test Chmbrs: 500-ml cups/~~beakers~~ (3) & 2.5 gallon aquaria
Photoperiod:	16 h light : 8 h dark	Light intensity:	100-300 ft-c.
Test Solution Vol.:	250 - 3000 ml	Replicates per Treatment:	1
Length of Test:	14 days	Organisms per Replicate:	5
Type of Food and Quantity per Chamber:	(1) Grnd Tetrafin - variable	Feeding Frequency:	Monday, Wednesday, Friday

Test Substance Characterization Parameters and Frequency:

Hardness: Initiation Alkalinity: Initiation NH_3: Initiation TRC: NA

pH: Init and Term Conductivity: Init and Term

Test Concentrations (Volume:Volume): 0 (Mod Hard), 95, 239, 597, 1492, 3730, 5595 mg/L NTO (Nominal)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: NA to NA IC_{25}: NA

Hist. 95% Control Limits: NA to NA Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

This is an extra set of NTO Test Treatments, the purpose of which is to collect growth data on Day 14 of the test. All surviving organisms in each test chamber will be euthanized with MS-222 and meaured on Day 14 of the study.

Study Director Initials: DAP Date: 5/20/15

(1) Feeding will commence after external gill closure @ ~Gosner Stage 25. See Protocol for amounts. DAP 5/20/15

(2) DAP 5/20/15-E (3) Plastic cups used for 1st week of test. DAP 10/15/15

(4) DAP 12/15/15-E

TEST SUBSTANCE USAGE LOG

Project Number: 15001-468-010 (Sacf. Reps)

QA: AR 10/23/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: @ NA	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (Grab or Comp)				
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	11644	11658		
Concurrent Control Water RW#	NA			
Date(s) Used	5/20/15	5/27/15		
		(Day 7)		

Preparation of Test Solutions (1)

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	1500	1500						
1.28%	19.2	1480.8	1500						
3.2%	48	1452	1500						
8%	120	1380	1500						
20%	300	1200	1500						
50%	750	750	1500						
75%	1125	375	1500						
Total	2362.2	8137.8	10500						

Initials / Date	DAP 5/20/15		
Initials / Date			
Initials / Date	See spreadsheet		
Initials / Date	for additional		
Initials / Date	mixing volumes		
Initials / Date	DAP 5/27/15		
Initials / Date			
Initials / Date			

(1) Mixed with solutions used in Definitive (Full Metamorphosis) test. DAP 5/20/15 (2) DAP 12/15/15

QA Form No. 060
Revision 3
Effective 02/14

① North. Leopard Frog (Lithobates pipiens)

~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 (Sacf. Reps)

QA: MR 10/23/15

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
MH	A	5	5	5	5		5		5	
0										
mg/L										
NTU										
1.28%	A	5	5	5	5		5		5	
201										
mg/L										
NTU										
3.2%	A	5	5	5	5		5		5	
480										
mg/L										
NTU										
8%	A	5	5	5	5		5		4	
1230										
mg/L										
NTU										
20.0%	A	5	5	5	5		5		5	
3070										
mg/L										
NTU										
50.0%	A	5	5	5	5		5		4	
7775										
mg/L										
NTU										
75.0%	A	5	5	0	–		—			Very weak. Day 1
11350										
mg/L										
NTU										
Date:		5/20/15	5/21/15	5/22/15	5/23/15		5/25/15		5/27/15	
Time:		1330	1500	1640	1055		1050		1440	
Initials:		DAP	DAP	DAP	DAP		DAP		DAP	

① DAP 6/30/15 E ② DAP 12/15/15 E

② North. Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 (Sacf. Reps)

QA: AR(10/23/15)

%Conc.	Test Replicate	Number of Surviving Organisms Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Remarks
MH	A	5	5		~~5~~ ①	5	5	5		
1.28%	A	5	5		~~5~~ ①	5	5	5		
3.2%	A	4	4			4	4	4		
8%	A	4	4			4	4	4		
20.0%	A	5	5			5	5	5		
50.0%	A	3	3			3	3	3		
75.0%	A	—	—	—	—	—	—	—		
Date:		5/28/15	5/29/15			6/1/15	6/2/15	6/3/15		
Time:		1645	1400			1405	1540	1050		
Initials:		DAP	DAP			DAP	DAP	AM		

① DAP 6/1/15 UP ② DAP 6/30/15 E ↑ Terminated
③ DAP 12/15/15 E

DAILY TOXICITY TEST LOG

QA: AR 10/23/15

Project Number:	15001-468-010 (Sacf. Reps)
Test Species:	~~Rana~~ pipiens ② Lithobates

General Comments	Random Chart: _____ Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 0	Test Solution Mixed at: 1000-1100 Test Organisms Added at: 1330 Temp: 24°C Range: 24-25°C	Fed @	DAP 5/20/15
Test Day 1	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DAP 5/21/15
Test Day 2	Real Time: 24 °C Min-Max Range: 24-26 °C Aeration initiated @ 1700	Fed @ 1650 ~0.025g/chmbr	DAP 5/22/15
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C DOs: Control: 5.7 50%: 5.7 Meter # 6	Fed @	DAP 5/23/15
Test Day 4	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @ ①~~1055~~	DAP 5/24/15
Test Day 5	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @ 1055 ~0.045g/chmbr	DAP 5/25/15
Test Day 6	Real Time: 25 °C Min-Max Range: 24-28 °C	Fed @	DAP 5/26/15
Test Day 7	Real Time: 26 °C Min-Max Range: 24-29 °C	Fed @ 1445 0.077g	DAP 5/27/15
Test Day 8	Real Time: 26 °C Min-Max Range: 24-28 °C	Fed @	DAP 5/28/15

① DAP 5/25/15 WP ② DAP 6/30/15 E ③ DAP 12/15/15 E

DAILY TOXICITY TEST LOG

Project Number: 15001-468-010 (Sacf. Reps) QA: AR 10/23/15

Test Species: ~~Rana~~ pipiens (2) Lithobates

General Comments	Random Chart: _____ Min/Max Thermometer # M-15		Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 26 °C	Min-Max Range: 24-28 °C	Fed @ 1120	DAP 5/29/15
Test Day 10	Real Time: 26 °C	Min-Max Range: 24-27 °C	Fed @	DAP 5/30/15
Test Day 11	Real Time: 26 °C	Min-Max Range: 24-26 °C	Fed @	DAP 5/31/15
Test Day 12	Real Time: 26 °C	Min-Max Range: 24-26 °C	Fed @ 1410 0.077g	DAP 6/1/15
Test Day 13	Real Time: 25 °C	Min-Max Range: 24-26 °C	Fed @	DAP 6/2/15
Test Day 14	Real Time: 26 °C Width & SVL measured on surviving tad poles (DAP)	Min-Max Range: 25-28 °C	Fed @ ~~1520~~ (1) Not Fed	DAP 6/3/15
Test Day 15	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 16	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 17	Real Time: °C	Min-Max Range: °C	Fed @	

(1) DAP 6/3/15 E (2) DAP 6/3d/15 E (3) DAP 12/15/15 E

Tadpole Sublethal Measurements

QA: AR 10/23/15

Test Number:	15001-468-010(14-D)	Test Description:	NTO 1-Generation (14-D)
Test Dates:	5/20/15 - 6/3/15	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Means width	Means SVL	Comments
Control	A	①~~9~~8.5	16		7.6	14	
	A	7	13				
	A	6.5	13				
	A	8	14				
	A	8	14				
1.28%	A	5	10		6.3	11.6	
	A	①~~6~~5	11				
	A	6.5	11				
	A	7	13				
	A	8	13				
3.2%	A	7.5	13		7.125	12.75	
	A	7.5	13				
	A	5.5	11				
	A	8	14				
8%	A	6.5	11		7.125	12.25	
	A	7.5	13				
	A	6.5	12				
	A	8	13				
Date		6/3/15 →					
Time		1330-1500 →					
Inits		DAP →					

① DAP 6/3/15 E ② DAP 12/15/15 E

Tadpole Sublethal Measurements

QA: AE 10/23/15

Test Number:	15001-468-010 (14-D)	Test Description:	NTO-1 Generation (14-D)
Test Dates:	5/20/15 - 6/3/15	Test Species:	Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	Eye Width (mm)	Means Width	Means SVL	Comments
20%	A	6.5	11.5		6.7	12.2	
	A	7	13				
	A	6.5	11.5				
	A	7	13				
	A	6.5	12				
50%	A	5.5	10		5.3	10	
	A	5	10				
	A	5.5	10				
Date		6/3/15	→				
Time		1330-1500	→				
Inits		DSP	→				

① DSP 12/15/15 E

CETIS Analytical Report

14①
Lithobates pipiens ~~28~~-Day ①

Report Date: 15 Oct-15 11:14 (p 1 of 2)
Test Code: 468-010-14d | 08-3470-7126

~~Fathead Minnow 48-h~~ Acute Survival Test 14-Day ①

TRE Environmental Strategies, LLC

Analysis ID:	07-7318-4920	**Endpoint:**	~~24h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:13	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Batch ID:	02-1168-5132	**Test Type:**	Survival ~~(48h)~~ (14 Day) ①	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-012 (2002)~~	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	**Species:**	~~Pimephales promelas~~ Lithobates pipiens	**Brine:**	~24 h Post-Hatch
Duration:	13d 21h	**Source:**	Carolina Biological Supply ①	**Age:**	~~48~~ h ①

Sample ID:	00-4076-3048	**Code:**	26DFEA8	**Client:**	Internal Lab
Sample Date:	~~19 May-15~~ ①	**Material:**	~~Not Applicable~~ NTO ①	**Project:**	Special Studies
Receive Date:		**Source:**	~~Well Water~~ ①		
Sample Age:	~~38h~~ NA ①	**Station:**			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
24	-13.52	41.04	32.87	3.759	0.7009	0.4393				Lack of Fit Not Tested

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	404.1	0.8764	1243
LC10	726.4	7.595	1892
LC15	1079	31.36	2611
LC20	1477	92.56	3529
LC25	1935	221.3	4836
LC40	3818	1241	17160
LC50	5747	2328	55250

14-Day Test
14-D LC50

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	2.55E-07	0.000226	-0.00044	0.000443	0.00113	0.9992	Non-Significant Parameter
Slope	1.427	0.5376	0.3731	2.48	2.654	0.0567	Non-Significant Parameter
Intercept	-5.364	1.909	-9.104	-1.623	-2.81	0.0483	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	12.73866	12.73866	1	6.702	0.0608	Non-Significant
Residual	7.603224	1.900806	4			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	7.603	9.488	0.1072	Non-Significant Heterogenity
	Likelihood Ratio GOF	10.3	9.488	0.0357	Significant Heterogenity
Distribution	Shapiro-Wilk W Normality	0.9664	0.6146	0.8714	Normal Distribution

① ~~24h~~ 14D Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	1	1	1	1	0	0	0.0%	0.0%	5	5
201		1	1	1	1	0	0	0.0%	0.0%	5	5
480		1	0.8	0.8	0.8	0	0	0.0%	20.0%	4	5
1230		1	0.8	0.8	0.8	0	0	0.0%	20.0%	4	5
3070		1	1	1	1	0	0	0.0%	0.0%	5	5
7775		1	0.6	0.6	0.6	0	0	0.0%	40.0%	3	5
11350		1	0	0	0	0	0		100.0%	0	5

① DAP 10/15/15 E ② DAP 12/15/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day (1)

~~Fathead Minnow 48-h~~ Acute Survival Test 14-Day

Report Date:	15 Oct-15 11:14 (p 2 of 2)
Test Code:	468-010-14d | 08-3470-7126

(1)

TRE Environmental Strategies, LLC

Analysis ID:	07-7318-4920	**Endpoint:**	~~24h~~ Survival Rate (1)	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:13	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

24h Survival Rate Detail

C-mg/L	Control Type	Rep 1
0	Dilution Water	1
201		1
480		0.8
1230		0.8
3070		1
7775		0.6
11350		0

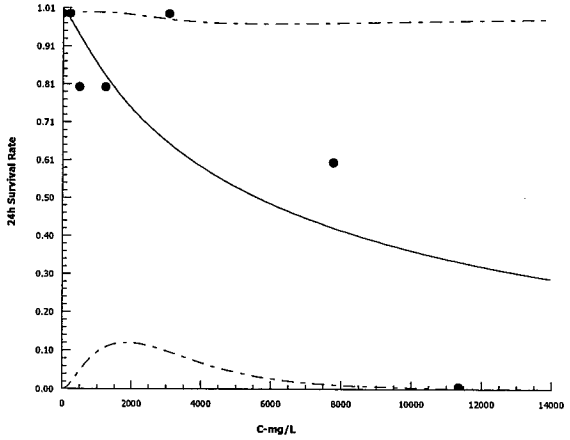
(1)

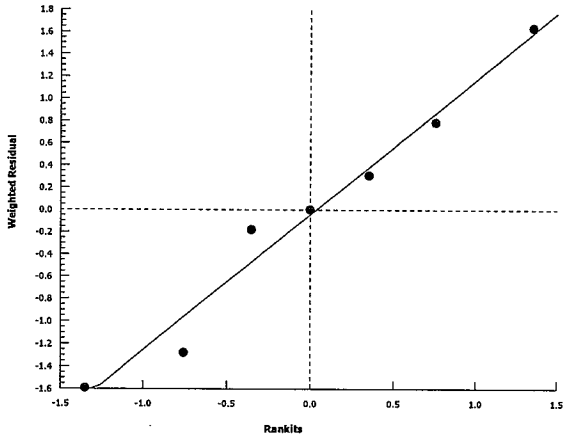
~~24h~~ 14-Day Survival Rate Binomials

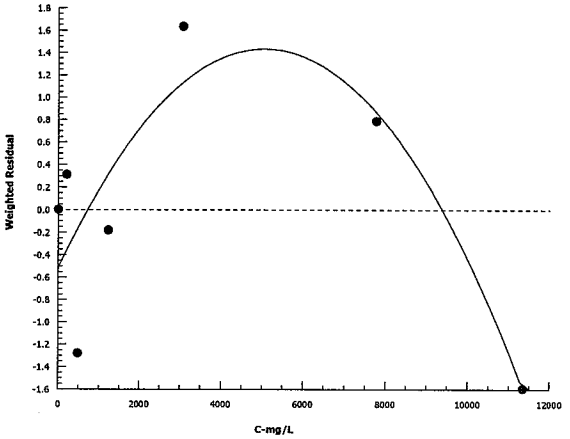
C-mg/L	Control Type	Rep 1
0	Dilution Water	5/5
201		5/5
480		4/5
1230		4/5
3070		5/5
7775		3/5
11350		0/5

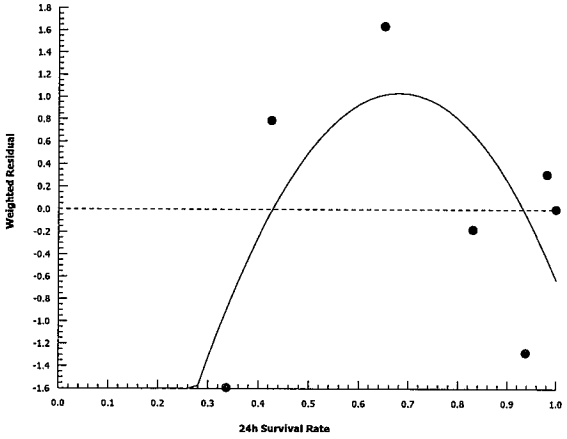
Graphics

Log-Normal [NED=A+B*log(X)]









(1) DAP 12/15/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day (1)

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	15 Oct-15 14:50 (p 1 of 2) (2)
Test Code:	468-010-14svl | 02-9163-6722
	TRE Environmental Strategies, LLC

Analysis ID:	08-1899-3273	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL, mm (1)	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:50	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Batch ID:	16-2471-9213	**Test Type:**	Survival-Development-Growth	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-013 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	**Species:**	~~Pimephales promelas~~ Lithobates pipiens (1)	**Brine:**	
Duration:	13d 21h	**Source:**	Carolina Biological Supply	**Age:**	~24 h post hatch ~~48 h~~ (1)

Sample ID:	16-2577-6304	**Code:**	60E760B0	**Client:**	
Sample Date:	~~19 Jun-15~~ (1)	**Material:**	~~Not Applicable~~ NTO (1)	**Project:**	Special Studies
Receive Date:	~~24 Jun-15 17:48~~ (1)	**Source:**	~~Reference Toxicant~~ (1)		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	14.1%	<201	201	NA	

Bonferroni Adj t Test ↑ 1-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201*	3.539	2.53	1.715	8	0.0052	CDF	Significant Effect
		480	1.738	2.53	1.819	7	0.2441	CDF	Non-Significant Effect
		1230	2.433	2.53	1.819	7	0.0613	CDF	Non-Significant Effect
		3070*	2.654	2.53	1.715	8	0.0381	CDF	Significant Effect
		7775*	5.108	2.53	1.98	6	0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	33.61538	6.723077	5	5.846	0.0017	Significant Effect
Error	23	1.15	20			
Total	56.61538		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.8556	4.44	0.5312	Equal Variances
Variances	Levene Equality of Variance	1.625	4.1	0.1991	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9786	0.891	0.8420	Normal Distribution

~~Mean Dry Biomass-mg~~ **Summary** Snout to Vent Length (mm) (1)

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	14	12.48	15.52	14	13	16	0.5477	8.75%	0.0%
201		5	11.6	9.934	13.27	11	10	13	0.6	11.6%	17.1%
480		4	12.75	10.75	14.75	13	11	14	0.6292	9.87%	8.93%
1230		4	12.25	10.73	13.77	12.5	11	13	0.4787	7.82%	12.5%
3070		5	12.2	11.26	13.14	12	11.5	13	0.3391	6.22%	12.9%
7775		3	10	10	10	10	10	10	0	0.0%	28.6%

~~Mean Dry Biomass-mg~~ **Detail** Snout to Vent Length (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	16	13	13	14	14
201		10	11	11	13	13
480		13	13	11	14	
1230		11	13	12	13	
3070		11.5	13	11.5	13	12
7775		10	10	10		

(1) DAP 10/15/15 E (2) DAP 12/15/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day ①

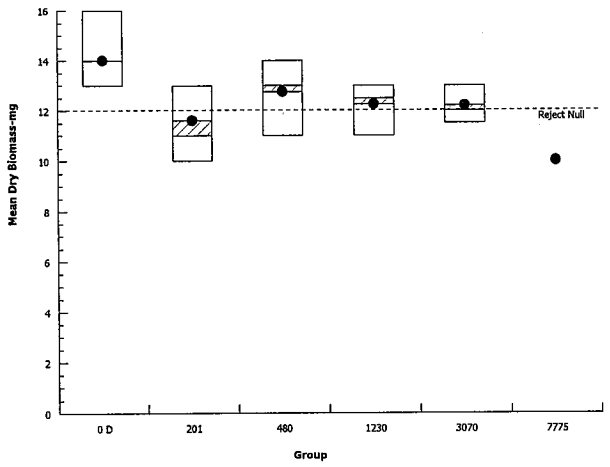
Report Date:	15 Oct-15 14:50 (p 2 of 2)
Test Code:	468-010-14svl | 02-9163-6722

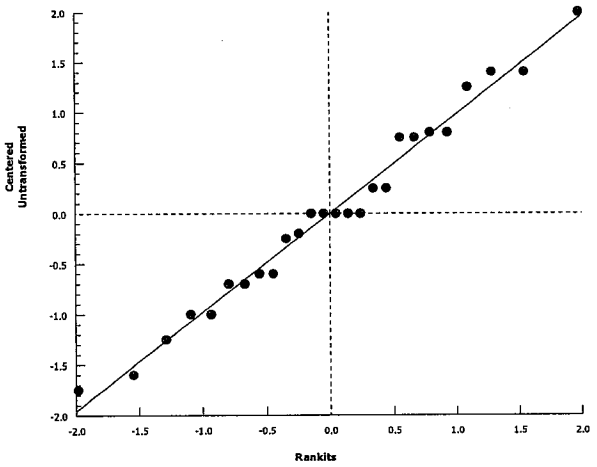
~~Fathead Minnow 7 d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	08-1899-3273	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL, mm ①	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:50	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Graphics





① DAP 10/15/15 E

Analyst: DAP QA: AR 10/23/15

CETIS Analytical Report

Lithobates pipiens 14-Day ①

Report Date: 15 Oct-15 14:51 (p 1 of 2)
Test Code: 468-010-14svl | 02-9163-6722

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	20-9768-1086	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL, mm	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:51	**Analysis:**	Parametric-Multiple Comparison ①	**Official Results:**	Yes

Batch ID:	16-2471-9213	**Test Type:**	Survival-Development-Growth	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-013 (2002)~~ ①	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	**Species:**	~~Pimephales promelas~~ Lithobates pipiens	**Brine:**	~24 h post hatch
Duration:	13d 21h	**Source:**	Carolina Biological Supply	**Age:**	~~48~~ h ①

Sample ID:	16-2577-6304	**Code:**	60E760B0	**Client:**	
Sample Date:	~~19 Jun-15~~ ①	**Material:**	~~Not Applicable~~ N TO ①	**Project:**	Special Studies
Receive Date:	~~24 Jun-15 17:48~~ ①	**Source:**	~~Reference Toxicant~~ ①		
Sample Age:	NA	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C <> T	NA	NA	15.9%	<201	201	NA	

Bonferroni Adj t Test ↑ 2-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201*	3.539	2.85	1.93	8	0.0103	CDF	Significant Effect
		480	1.738	2.85	2.047	7	0.4883	CDF	Non-Significant Effect
		1230	2.433	2.85	2.047	7	0.1225	CDF	Non-Significant Effect
		3070	2.654	2.85	1.93	8	0.0762	CDF	Non-Significant Effect
		7775*	5.108	2.85	2.228	6	0.0003	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	33.61538	6.723077	5	5.846	0.0017	Significant Effect
Error	23	1.15	20			
Total	56.61538		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.8556	4.44	0.5312	Equal Variances
Variances	Levene Equality of Variance	1.625	4.1	0.1991	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9786	0.891	0.8420	Normal Distribution

~~Mean Dry Biomass-mg~~ **Summary** Snout to Vent Length (mm) ①

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	14	12.48	15.52	14	13	16	0.5477	8.75%	0.0%
201		5	11.6	9.934	13.27	11	10	13	0.6	11.6%	17.1%
480		4	12.75	10.75	14.75	13	11	14	0.6292	9.87%	8.93%
1230		4	12.25	10.73	13.77	12.5	11	13	0.4787	7.82%	12.5%
3070		5	12.2	11.26	13.14	12	11.5	13	0.3391	6.22%	12.9%
7775		3	10	10	10	10	10	10	0	0.0%	28.6%

~~Mean Dry Biomass-mg~~ **Detail** Snout to Vent Length (mm) ①

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	16	13	13	14	14
201		10	11	11	13	13
480		13	13	11	14	
1230		11	13	12	13	
3070		11.5	13	11.5	13	12
7775		10	10	10		

① DLP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day ①

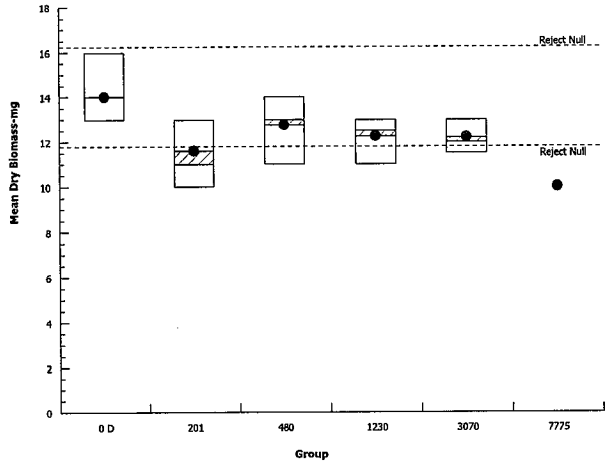
Report Date:	15 Oct-15 14:51 (p 2 of 2)
Test Code:	468-010-14svl | 02-9163-6722

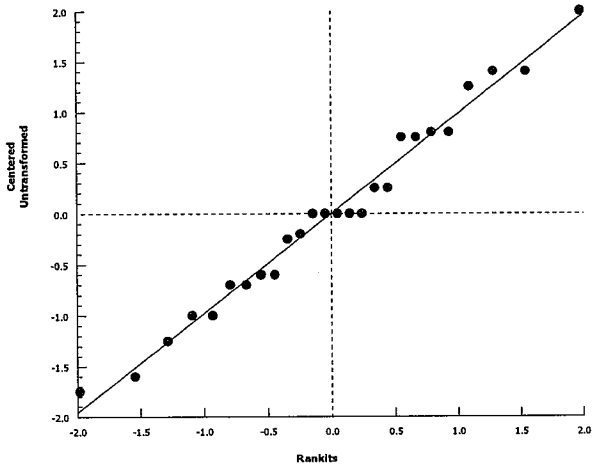
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	20-9768-1086	Endpoint:	~~Mean Dry Biomass-mg~~ SVL, mm ①	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 14:51	Analysis:	Parametric-Multiple Comparison	Official Results:	Yes

Graphics





① DAP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day (1)

Report Date:	15 Oct-15 14:52 (p 1 of 2)		
Test Code:	468-010-14svl | 02-9163-6722		

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	09-7855-3809	Endpoint:	~~Mean Dry Biomass-mg~~ SVL, mm	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 14:51	Analysis:	Linear Interpolation (ICPIN) (1)	Official Results:	Yes

Batch ID:	16-2471-9213	Test Type:	Survival-Development-Growth	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-013 (2002)~~ (1)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	Species:	~~Pimephales promelas~~ Lithobates pipiens (1)	Brine:	~24 h post hatch
Duration:	13d 21h	Source:	Carolina Biological Supply	Age:	~~48 h~~ (1)

Sample ID:	16-2577-6304	Code:	60E760B0	Client:	
Sample Date:	~~19 Jun-15~~ (1)	Material:	~~Not Applicable~~ NTO (1)	Project:	Special Studies
Receive Date:	~~24 Jun-15 17:48~~ (1)	Source:	~~Reference Toxicant~~ (1)		
Sample Age:	NA	Station:			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	942293	200	Yes	Two-Point Interpolation

Point Estimates

Level		95% LCL	95% UCL
IC5	6.88	1.199	902.9
IC10	61.09	N/A	5145
IC15	3485	N/A	5178
IC20	4684	2344	7119
IC25	6295	3488	N/A
IC40	>7775	N/A	N/A
IC50	>7775	N/A	N/A

14-Day IC25 on Snout-to-Vent Length (SVL) (1)

~~Mean Dry Biomass-mg~~ Summary — Snout to Vent Length (mm) — Calculated Variate

Group	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	5	14	13	16	0.5477	1.225	8.75%	0.0%
201		5	11.6	10	13	0.6	1.342	11.6%	17.1%
480		4	12.75	11	14	0.6292	1.258	9.87%	8.93%
1230		4	12.25	11	13	0.4787	0.9574	7.82%	12.5%
3070		5	12.2	11.5	13	0.3391	0.7583	6.22%	12.9%
7775		3	10	10	10	0	0	0.0%	28.6%

~~Mean Dry Biomass-mg~~ Detail — Snout to Vent Length (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	16	13	13	14	14
201		10	11	11	13	13
480		13	13	11	14	
1230		11	13	12	13	
3070		11.5	13	11.5	13	12
7775		10	10	10		

(1) DAP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day ①

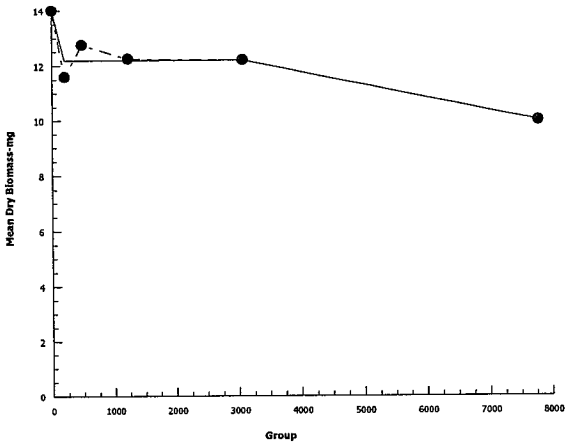
Report Date:	15 Oct-15 14:52 (p 2 of 2)
Test Code:	468-010-14svl | 02-9163-6722

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	09-7855-3809	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL, mm ①	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



① DAP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens ~~28~~ 14-Day (1)

Report Date: 15 Oct-15 14:54 (p 1 of 2)
Test Code: 468-010-14wid | 15-5228-4309

~~Fathead Minnow 7-d Larval~~ Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	15-8753-7082	**Endpoint:**	~~Mean Dry Biomass-mg~~ Body width, mm	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:53	**Analysis:**	Parametric-Multiple Comparison (1)	**Official Results:**	Yes

Batch ID:	16-2471-9213	**Test Type:**	Survival-Development-Growth	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-013 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	**Species:**	~~Pimephales promelas~~ Lithobates pipiens (1)	**Brine:**	~24 h post hatch
Duration:	13d 21h	**Source:**	Carolina Biological Supply	**Age:**	~~48~~ h (1)

Sample ID:	19-3786-4197	**Code:**	73817605	**Client:**	Internal Lab
Sample Date:	~~19 May-15~~ (1)	**Material:**	~~Not Applicable~~ WTO (1)	**Project:**	Special Studies
Receive Date:		**Source:**	~~Reference Toxicant~~ (1)		
Sample Age:	~~38h~~ (1)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	21.3%	3070	7775	4886	

Bonferroni Adj t Test ↑ 1-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	2.347	2.53	1.4	8	0.0734	CDF	Non-Significant Effect
		480	0.8085	2.53	1.485	7	1.0000	CDF	Non-Significant Effect
		1230	0.8085	2.53	1.485	7	1.0000	CDF	Non-Significant Effect
		3070	1.625	2.53	1.4	8	0.2997	CDF	Non-Significant Effect
		7775*	3.544	2.53	1.617	6	0.0051	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	11.77372	2.354743	5	3.07	0.0324	Significant Effect
Error	15.34167	0.7670833	20			
Total	27.11538		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.413	15.1	0.0937	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.961	0.891	0.4107	Normal Distribution

~~Mean Dry Biomass-mg~~ **Summary** Body width (mm) (1)

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	7.6	6.58	8.62	8	6.5	8.5	0.3674	10.8%	0.0%
201		5	6.3	4.681	7.919	6.5	5	8	0.5831	20.7%	17.1%
480		4	7.125	5.361	8.889	7.5	5.5	8	0.5543	15.6%	6.25%
1230		4	7.125	5.932	8.318	7	6.5	8	0.375	10.5%	6.25%
3070		5	6.7	6.36	7.04	6.5	6.5	7	0.1225	4.09%	11.8%
7775		3	5.333	4.616	6.05	5.5	5	5.5	0.1667	5.41%	29.8%

~~Mean Dry Biomass-mg~~ **Detail** Body width (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	8.5	7	6.5	8	8
201		5	5	6.5	7	8
480		7.5	7.5	5.5	8	
1230		6.5	7.5	6.5	8	
3070		6.5	7	6.5	7	6.5
7775		5.5	5	5.5		

(1) DAP 10/16/15 E

CETIS Analytical Report

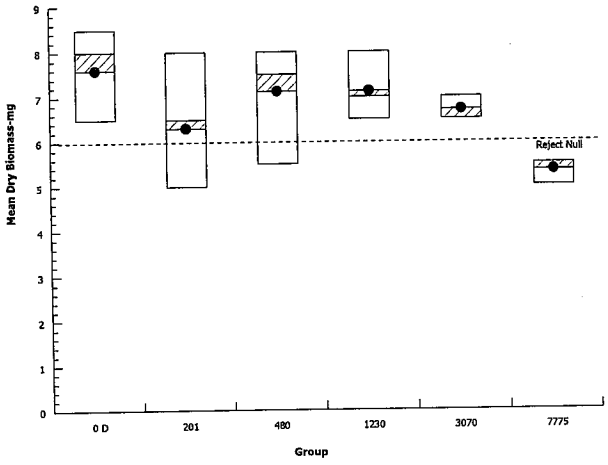
Lithobates pipiens 14-Day ①

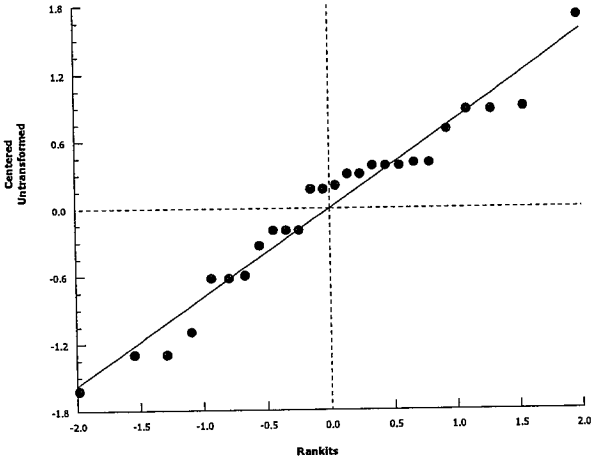
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	15 Oct-15 14:54 (p 2 of 2)
Test Code:	468-010-14wid | 15-5228-4309
TRE Environmental Strategies, LLC	

Analysis ID:	15-8753-7082	**Endpoint:**	~~Mean Dry Biomass-mg~~ Body width, mm ①	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:53	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Graphics





① DAP 10/16/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day (1)

Report Date:	15 Oct-15 14:54 (p 1 of 2)
Test Code:	468-010-14wid | 15-5228-4309

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	10-5068-7061	Endpoint:	~~Mean Dry Biomass-mg~~ Body width, mm	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 14:54	Analysis:	Parametric-Multiple Comparison (1)	Official Results:	Yes

Batch ID:	16-2471-9213	Test Type:	Survival-Development-Growth	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-013 (2002)~~ (1)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	Species:	~~Pimephales promelas~~ Lithobates pipiens (1)	Brine:	~24 h post-hatch
Duration:	13d 21h	Source:	Carolina Biological Supply	Age:	~~48~~ h (1)

Sample ID:	19-3786-4197	Code:	73817605	Client:	Internal Lab
Sample Date:	~~19 May-15~~ (1)	Material:	~~Not Applicable~~ NTO (1)	Project:	Special Studies
Receive Date:		Source:	~~Reference Toxicant~~ (1)		
Sample Age:	~~38h~~ (1)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C <> T	NA	NA	23.9%	3070	7775	4886	

Bonferroni Adj t Test ↑ 2-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	2.347	2.85	1.576	8	0.1467	CDF	Non-Significant Effect
		480	0.8085	2.85	1.672	7	1.0000	CDF	Non-Significant Effect
		1230	0.8085	2.85	1.672	7	1.0000	CDF	Non-Significant Effect
		3070	1.625	2.85	1.576	8	0.5993	CDF	Non-Significant Effect
		7775*	3.544	2.85	1.82	6	0.0102	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	11.77372	2.354743	5	3.07	0.0324	Significant Effect
Error	15.34167	0.7670833	20			
Total	27.11538		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.413	15.1	0.0937	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.961	0.891	0.4107	Normal Distribution

~~Mean Dry Biomass-mg~~ Summary ~~Both~~ Body width (mm) (1)

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	7.6	6.58	8.62	8	6.5	8.5	0.3674	10.8%	0.0%
201		5	6.3	4.681	7.919	6.5	5	8	0.5831	20.7%	17.1%
480		4	7.125	5.361	8.889	7.5	5.5	8	0.5543	15.6%	6.25%
1230		4	7.125	5.932	8.318	7	6.5	8	0.375	10.5%	6.25%
3070		5	6.7	6.36	7.04	6.5	6.5	7	0.1225	4.09%	11.8%
7775		3	5.333	4.616	6.05	5.5	5	5.5	0.1667	5.41%	29.8%

~~Mean Dry Biomass-mg~~ Detail Body width (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	8.5	7	6.5	8	8
201		5	5	6.5	7	8
480		7.5	7.5	5.5	8	
1230		6.5	7.5	6.5	8	
3070		6.5	7	6.5	7	6.5
7775		5.5	5	5.5		

(1) DSP 10/16/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day ①

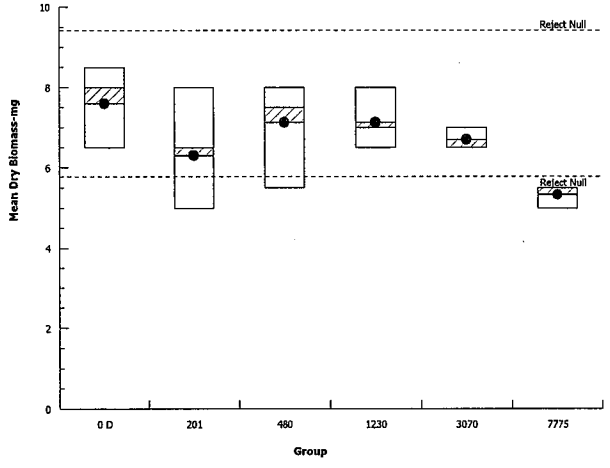
Report Date:	15 Oct-15 14:54 (p 2 of 2)
Test Code:	468-010-14wid | 15-5228-4309

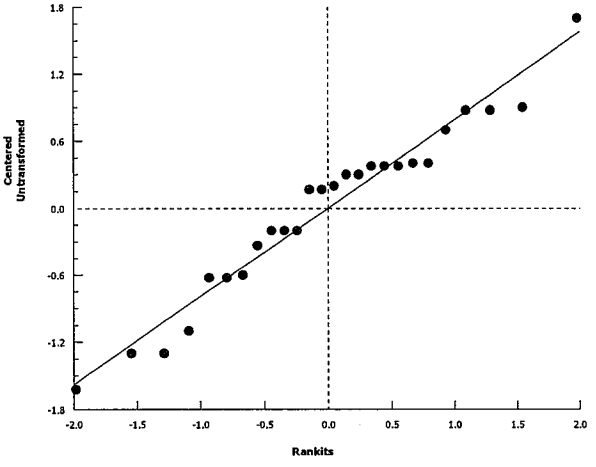
~~Fathead Minnow 7-d L~~arval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	10-5068-7061	**Endpoint:**	~~Mean Dry Biomass-mg~~ Body width, mm	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 14:54	**Analysis:**	Parametric-Multiple Comparison ①	**Official Results:**	Yes

Graphics





① DAP 10/16/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day ①

Report Date:	15 Oct-15 14:54 (p 1 of 2)
Test Code:	468-010-14wid | 15-5228-4309

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	00-6888-0916	Endpoint:	~~Mean Dry Biomass-mg~~ Body width, mm	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 14:54	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Batch ID:	16-2471-9213	Test Type:	Survival-Development-Growth	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-013 (2002)~~	Diluent:	Mod-Hard Synthetic Water
Ending Date:	03 Jun-15 10:50	Species:	~~Pimephales promelas~~ Lithobates pipiens ①	Brine:	~24 h post-hatch
Duration:	13d 21h	Source:	Carolina Biological Supply	Age:	~~48 h~~

Sample ID:	19-3786-4197	Code:	73817605	Client:	Internal Lab
Sample Date:	~~19 May-15~~ ①	Material:	~~Not Applicable~~ N TOX	Project:	Special Studies
Receive Date:		Source:	~~Reference Toxicant~~ ①		
Sample Age:	~~38h~~ ①	Station:			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1209281	200	Yes	Two-Point Interpolation

Point Estimates

Level		95% LCL	95% UCL
IC5	13.72	N/A	4963
IC10	1307	N/A	5467
IC15	3614	N/A	5861
IC20	4680	2386	7389
IC25	6059	3377	N/A
IC40	>7775	N/A	N/A
IC50	>7775	N/A	N/A

14-Day IC25 on Body width

~~Mean Dry Biomass-mg~~ Summary Body width (mm) Calculated Variate

Group	Control Type	Count	Mean	Min ①	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	5	7.6	6.5	8.5	0.3674	0.8216	10.8%	0.0%
201		5	6.3	5	8	0.5831	1.304	20.7%	17.1%
480		4	7.125	5.5	8	0.5543	1.109	15.6%	6.25%
1230		4	7.125	6.5	8	0.375	0.75	10.5%	6.25%
3070		5	6.7	6.5	7	0.1225	0.2739	4.09%	11.8%
7775		3	5.333	5	5.5	0.1667	0.2887	5.41%	29.8%

~~Mean Dry Biomass-mg~~ Detail Body width (mm) ①

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	8.5	7	6.5	8	8
201		5	5	6.5	7	8
480		7.5	7.5	5.5	8	
1230		6.5	7.5	6.5	8	
3070		6.5	7	6.5	7	6.5
7775		5.5	5	5.5		

① DAP 10/16/15 E

CETIS Analytical Report

Lithobates pipiens 14-Day (1)

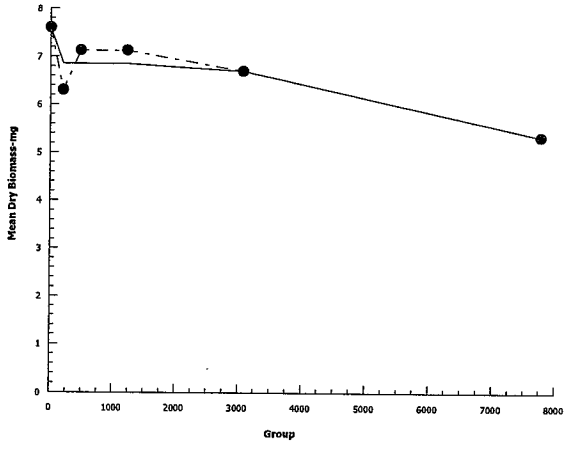
Report Date:	15 Oct-15 14:54 (p 2 of 2)		
Test Code:	468-010-14wid | 15-5228-4309		

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	00-6888-0916	Endpoint:	~~Mean Dry Biomass-mg~~ Body Width, mm (1)	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 14:54	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Graphics



(1) DSP 10/16/15 E

QA Form No. 051
Revision 5
Effective 02/14

QA: AR 10/23/15

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Chronic

Project Number: 15001-468-010 (Sacf. Reps)

Test Substance: NTO - pH-adjusted

Species: *Rana pipiens*

Dilution Water: Mod Hard

Organism Lot or Batch Number: 15-018

Concurrent Control Water: NA

Age: ~24 h Post-hatch (24-72 h)

Supplier: Carolina Biological

Date and Time Test Began: 5/20/15 @ 1330

Date and Time Test Ended: 6/17/15 @ 1035

Protocol Number:

Investigator(s): DAP / AB

Background Information

Type of Test: Static-Renewal

pH control?: Yes No

If yes, give % CO_2: NA

Test Temperature: (2) 22 ± ~~2~~ 3 °C

Env. Chmbr/Bath #: 1

Test Chmbrs: 500-ml cups/~~beakers~~ (3) & 2.5 gallon aquaria

Photoperiod: 16 h light : 8 h dark

Light intensity: 100-300 ft-c.

Test Solution Vol.: 250 - 3000 ml

Replicates per Treatment: 1

Length of Test: 28 days

Organisms per Replicate: 5

Type of Food and Quantity per Chamber: (1) Grnd Tetrafin - variable

Feeding Frequency: Monday, Wednesday, Friday

Test Substance Characterization Parameters and Frequency:

Hardness: Initiation Alkalinity: Initiation NH_3: Initiation TRC: NA

pH: Init and Term Conductivity: Init and Term

Test Concentrations (Volume:Volume): 0 (Mod Hard), 95, 239, 597, 1492, 3730, 5595 mg/L NTO (Nominal)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: NA to NA IC_{25}: NA

Hist. 95% Control Limits: NA to NA Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

This is an extra set of NTO Test Treatments, the purpose of which is to collect growth data on Day 28 of the test. All surviving organisms in each test chamber will be euthanized with MS-222 and meaured on Day 28 of the study.

Study Director Initials: DAP Date: 5/20/15

(1) Feeding will commence after external gill closure @ ~Gosner Stage 25. See Protocol for amounts. DAP 5/20/15

(2) DAP 5/20/15

(3) Plastic cups used for 1st week of Test. DAP 10/15/15

TEST SUBSTANCE USAGE LOG

Project Number: 15001-468-010 (Sacf. Reps)

QA: AR 10/23/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: NA @	From: @	From: @	From: @
	To: NA @	To: @	To: @	To: @
Sample Type (Grab or Comp)				
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	11644	11658	11668	11675
Concurrent Control Water RW#	NA			
Date(s) Used	5/20/15	5/27/15	6/3/15	6/10/15
		(Day 7)	(Day 14)	(Day 21)

Preparation of Test Solutions ①

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	1500	1500						
1.28%	19.2	1480.8	1500						
3.2%	48	1452	1500						
8%	120	1380	1500						
20%	300	1200	1500						
50%	750	750	1500						
75%	1125	375	1500						
Total	2362.2	8137.8	10500						

Initials / Date	DAP 5/20/15		
Initials / Date			
Initials / Date	New Solutions prepared on Days 7 (5/27) 14 (6/3) 21 (6/10),		
Initials / Date	28 (6/17), 35 (6/24), 42 (7/1) + 49 (7/8)		
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			

① Mixed with solutions used in Definitive (Full Metamorphosis) Test. DAP 5/20/15

QA Form No. 060
Revision 3
Effective 02/14

① Northern Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 (Sacf. Reps)

QA: ARC 10/23/15

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
MH	A	5	5	5	5		5		5	
1.28%	A	5	5	5	5		5		5	
201										
mg/L										
NTO										
3.2%	A	5	5	5	5		5		4	
480										
mg/L										
NTO										
8%	A	5	5	5	5		5		5	
1230										
mg/L										
NTO										
20.0%	A	5	5	5	5		5		5	
3070										
mg/L										
NTO										
50.0%	A	5	5	5	5		5		5	
7775										
mg/L										
NTO										
75.0%	A	5	5	0	–		–			Very weak - Day 1
11350										
mg/L										
NTO										
Date:		5/20/15	5/21/15	5/22/15	5/23/15		5/25/15		5/27/15	
Time:		1330	1500	1640	1055		1050		1440	
Initials:		DAP	DAP	DAP	DAP		DAP		DAP	

① DAP 6/30/15 E

① North. Leopard Frog (Lithobates pipiens)

~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 (Sacf. Reps)

QA: AR 10/23/15

%Conc.	Test Replicate	Number of Surviving Organisms								Remarks
		Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	
MH	A	5	5			5	5	5	5	
1.28%	A	5	5			5	5	5	5	
3.2%	A	4	4			4	4	4	4	
8%	A	4	4			4	4	4	4	
20.0%	A	5	5			5	5	5	5	
50.0%	A	5	5			4	4	4	4	
75.0%	A	—	—	—	—	—	—	—	—	
Date:		5/28/15	5/29/15			6/1/15	6/2/15	6/3/15	6/4/15	
Time:		1640	1355			1414	1545	1000	1535	
Initials:		DAP	DAP			DAP	DAP	AS	DAP	

① DAP 6/30/15 E

① North. Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 (Sacf. Reps) QA: AR 10/23/15

%Conc.	Test Replicate	Number of Surviving Organisms								Remarks
		Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	
MH	A	5			5	5	5	5	5	
1.28%	A	5			5	5	5	5	5	
3.2%	A	4			4	4	4	4	4	
8%	A	4			4	4	4	4	4	
20.0%	A	5			5	5	5	5	5	
50.0%	A	4			4	4	4	4	4	
75.0%	A									
Date:		6/5/15			6/8/15	6/9/15	6/10/15	6/11/15	6/12/15	
Time:		1455			1140	1653	1100	1715	1410	
Initials:		DAP			DAP	DAP	AB	DAP	AB	

① DAP 6/30/15 E

① North. Leopard Frog (Lithobates pipiens)
~~FATHEAD MINNOW (*PIMEPHALES PROMELAS*)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 (Sacf. Reps)

QA: AR 10/23/15

%Conc.	Test Replicate	Number of Surviving Organisms								
		Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Remarks
MH	A			5	5	5				
1.28%	A			5	5	5				
3.2%	A			4	4	4				
8%	A			4	4	4				
20.0%	A			5	5	5				
50.0%	A			3	3	3				
75.0%	A									
Date:				6/15/15	6/16/15	6/17/15				
Time:				1105	1116	1035				
Initials:				DSP	DSP	DSP				

↑ Terminated on 6/17/15 DSP

① DSP 6/30/15 E

DAILY TOXICITY TEST LOG

QA: AR 10/23/15

Project Number:	15001-468-010 (Sacf. Reps)
Test Species:	~~Rana pipiens~~ Lithobates (1)

General Comments	Random Chart: NA Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 0	Test Solution Mixed at: 1000-1100 Test Organisms Added at: 1330 Temp: 24°C Range: 24-25°C	Fed @	DAP 5/20/15
Test Day 1	Real Time: 24 °C Min-Max Range: 23-24 °C	Fed @	DAP 5/21/15
Test Day 2	Real Time: 24 °C Min-Max Range: 24-26 °C Aeration initiated @ 1700	Fed @ 1650 -0.025g/chmbr	DAP 5/22/15
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @	DAP 5/23/15
Test Day 4	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @	DAP 5/24/15
Test Day 5	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @ 1055 -0.045g	DAP 5/25/15
Test Day 6	Real Time: 25 °C Min-Max Range: 24-28 °C	Fed @	DAP 5/26/15
Test Day 7	Real Time: 26 °C Min-Max Range: 24-29 °C	Fed @ 1445 0.077g	DAP 5/27/15
Test Day 8	Real Time: 26 °C Min-Max Range: 24-28 °C	Fed @	DAP 5/28/15

(1) DAP 6/30/15 E

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010 (Sacf. Reps)	QA: AR 10/23/15
Test Species:	~~Rana~~ *pipiens* ① Lithobates	

General Comments	Random Chart: NA	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 26 °C	Min-Max Range: 24-28 °C	Fed @ 1120	DAP 5/29/15
Test Day 10	Real Time: 26 °C	Min-Max Range: 24-27 °C	Fed @	DAP 5/30/15
Test Day 11	Real Time: 26 °C	Min-Max Range: 24-26 °C	Fed @	DAP 5/31/15
Test Day 12	Real Time: 26 °C	Min-Max Range: 24-26 °C	Fed @ 1416 0.077g	DAP 6/1/15
Test Day 13	Real Time: 25 °C	Min-Max Range: 24-26 °C	Fed @	DAP 6/2/15
Test Day 14	Real Time: 26 °C	Min-Max Range: 25-28 °C	Fed @ 1520 0.1865g	DAP 6/3/15
Test Day 15	Real Time: 26 °C	Min-Max Range: 25-28 °C	Fed @	DAP 6/4/15
Test Day 16	Real Time: 24 °C	Min-Max Range: 24-26 °C	Fed @ 1535 0.1865g	DAP 6/5/15
Test Day 17	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/6/15

① DAP 6/30/15 E

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010 (Sacf. Reps)	QA: AR 10/23/15
Test Species:	~~Rana~~ pipiens ① Lithobates	

General Comments	Random Chart: NA	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 18	Real Time: 24 °C	Min-Max Range: 24-26 °C	Fed @	DAP 6/7/15
Test Day 19	Real Time: 25 °C	Min-Max Range: 24-26 °C	Fed @ 1225 0.1865g	DAP 6/8/15
Test Day 20	Real Time: 24 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/9/15
Test Day 21	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @ 1615 0.1865g	AB 6/10/15
Test Day 22	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/11/15
Test Day 23	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @ 1435 0.1865g	AB 6/12/15
Test Day 24	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/13/15
Test Day 25	Real Time: 24 °C	Min-Max Range: 24-27 °C	Fed @	DAP 6/14/15
Test Day 26	Real Time: 25 °C	Min-Max Range: 24-27 °C	Fed @ 1110 0.1865g	DAP 6/15/15

① DAP 6/30/15 E ② AB 12/15/15 E

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010 (Sacf. Reps)	QA: AR 10/23/15
Test Species:	~~Rana~~ *pipiens* ① Lithobates	

General Comments	Random Chart: NA Min/Max Thermometer # M-15		Feeding Grnd Tetramin	Initials/Date
Test Day 27	Real Time: 22 °C	Min-Max Range: 21-25 °C	Fed @	DAP 6/16/15
Test Day 28	Real Time: 22 °C Test terminated. Tadpoles euthanized + measured. DAP	Min-Max Range: 21-25 °C	Fed @	DAP 6/17/15
Test Day 29	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 30	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 31	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 32	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 33	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 34	Real Time: °C	Min-Max Range: °C	Fed @	
Test Day 35	Real Time: °C	Min-Max Range: °C	Fed @	

① DAP 6/30/15 E ② AB 11/15/15 E

Tadpole Sublethal Measurements

QA: AR 3/31/16

Test Number: 15001-468-010	Test Description: NTO 1-Generation Study
Test Dates: 5/20/15 - 6/17/15	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	GS ② ~~Eye Width (mm)~~	Means Width	Comments SVL
0		14	26	37	13	22.8
		9	20	32		
		12	23	36		
		11	20	34		
		19	25	37		
1.28		9	20	34	10.6	22.6
		9	20	33		
		10	22	35	Bent Spine	
		11	22	36		
		14	29	38		
3.2		14	26	36	11	22
		11	23	34		
		9	19	33		
		10	20	34		
8		14	29	37	11.25	23.75
		10	23	34		
		12	23	~~34~~ ① 35		
		9	20	33		
Date		6/17/15	→	→		
Time		1500-1630	→	→		
Inits		DAP	→	→		

① DAP 6/17/15 E ② DAP 6/17/15 CF

Tadpole Sublethal Measurements

QA: AR 3/31/16

Test Number: 15001-468-010	Test Description: NTO, 1-Generation Study
Test Dates: 5/20/15 - 6/17/15	Test Species: Rana pipiens

Treat/ Concn	Rep	Body Width (mm)	Body Length (S-V lngth) (mm)	~~Eye Width (mm)~~ GS ①	Means width	Comments SVL
20		10	23	35	10.8	23
		13	25	35		
		10	21	34		
		10	22	33		
		11	24	35		
50		8	15	30	8	15
		8	15	30		
		8	15	30		
Date		6/17/15	→	→		
Time		1500-1630	→	→		
Inits		DAP	→	→		

① DAP 6/17/15 CF

CETIS Analytical Report

Lithobates pipiens 28-Day ①

Report Date:	15 Oct-15 11:14 (p 1 of 2)
Test Code:	468-010-14d | 08-3470-7126

~~Fathead Minnow 48-h~~ Acute Survival Test 28-Day

TRE Environmental Strategies, LLC

Analysis ID:	15-8815-2397	Endpoint:	~~48h~~ Survival Rate ①	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 11:14	Analysis:	Linear Regression (MLE)	Official Results:	Yes

Batch ID:	02-1168-5132	Test Type:	Survival ~~(48h)~~ (28 Day) ①	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-012 (2002)~~	Diluent:	Mod-Hard Synthetic Water
Ending Date:	~~03 Jun-15 10:50~~ 17 June 2015 10:35 ①	Species:	~~Pimephales promelas~~ Lithobates pipiens ①	Brine:	~24 h post hatch ①
Duration:	~~13~~ 27d 21h ①	Source:	Carolina Biological Supply	Age:	~~48 h~~

Sample ID:	00-4076-3048	Code:	26DFEA8	Client:	Internal Lab
Sample Date:	~~19 May-15~~ ①	Material:	~~Not Applicable~~ NTO ①	Project:	Special Studies
Receive Date:		Source:	~~Well Water~~ ①		
Sample Age:	~~38h~~ NA ①	Station:			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
24	-13.52	41.04	32.87	3.759	0.7009	0.4393				Lack of Fit Not Tested

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	404.1	0.8764	1243
LC10	726.4	7.595	1892
LC15	1079	31.36	2611
LC20	1477	92.56	3529
LC25	1935	221.3	4836
LC40	3818	1241	17160
LC50	5747	2328	55250

28-Day Test
28-D LC50

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	2.55E-07	0.000226	-0.00044	0.000443	0.00113	0.9992	Non-Significant Parameter
Slope	1.427	0.5376	0.3731	2.48	2.654	0.0567	Non-Significant Parameter
Intercept	-5.364	1.909	-9.104	-1.623	-2.81	0.0483	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	12.73866	12.73866	1	6.702	0.0608	Non-Significant
Residual	7.603224	1.900806	4			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	7.603	9.488	0.1072	Non-Significant Heterogenity
	Likelihood Ratio GOF	10.3	9.488	0.0357	Significant Heterogenity
Distribution	Shapiro-Wilk W Normality	0.9664	0.6146	0.8714	Normal Distribution

① ~~48h~~ 28 D Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	1	1	1	1	0	0	0.0%	0.0%	5	5
201		1	1	1	1	0	0	0.0%	0.0%	5	5
480		1	0.8	0.8	0.8	0	0	0.0%	20.0%	4	5
1230		1	0.8	0.8	0.8	0	0	0.0%	20.0%	4	5
3070		1	1	1	1	0	0	0.0%	0.0%	5	5
7775		1	0.6	0.6	0.6	0	0	0.0%	40.0%	3	5
11350		1	0	0	0	0	0		100.0%	0	5

① DAP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-D ①

~~Fathead Minnow 48-h~~ Acute Survival Test

Report Date:	15 Oct-15 11:14 (p 2 of 2)
Test Code:	468-010-14d | 08-3470-7126

TRE Environmental Strategies, LLC

Analysis ID:	15-8815-2397	Endpoint:	~~48h~~ 28-Day Survival Rate ①	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 11:14	Analysis:	Linear Regression (MLE)	Official Results:	Yes

48h Survival Rate Detail

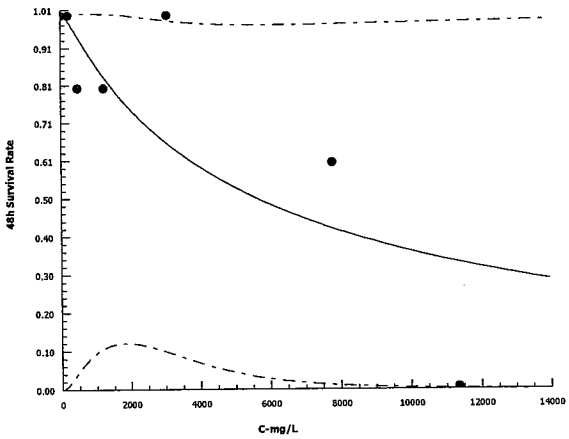
C-mg/L	Control Type	Rep 1
0	Dilution Water	1
201		1
480		0.8
1230		0.8
3070		1
7775		0.6
11350		0

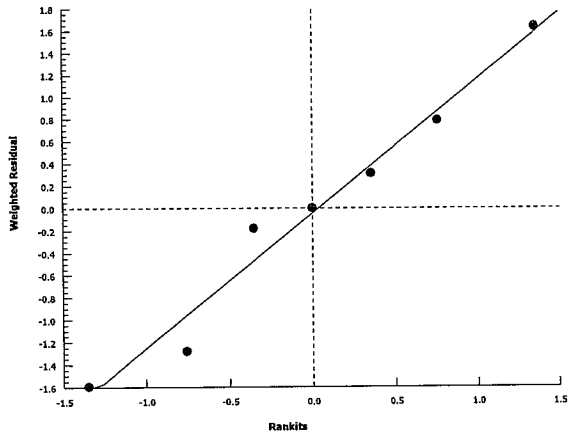
~~48h~~ 28-D Survival Rate Binomials ①

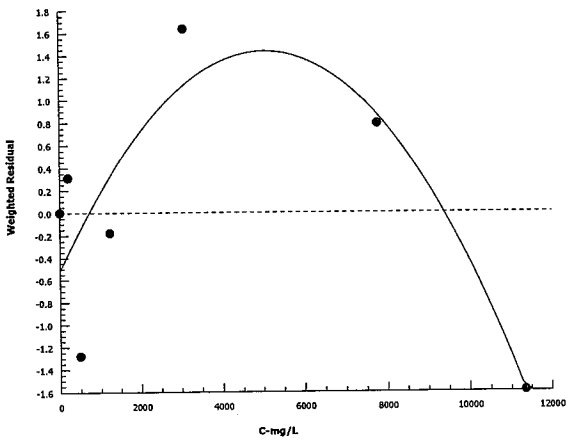
C-mg/L	Control Type	Rep 1
0	Dilution Water	5/5
201		5/5
480		4/5
1230		4/5
3070		5/5
7775		3/5
11350		0/5

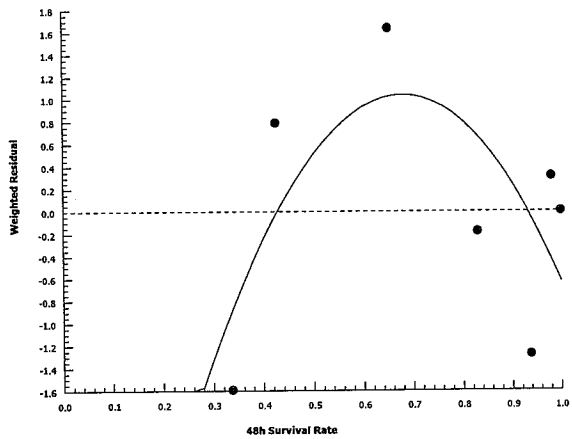
Graphics

Log-Normal [NED=A+B*log(X)]









CETIS Analytical Report

Lithobates pipiens 28-Day ①

Report Date:	15 Oct-15 11:00 (p 1 of 2)
Test Code:	468-010-28svl | 12-3899-7787

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	14-9396-3966	Endpoint:	~~Mean Dry Biomass-mg~~ SVL, mm ①	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 10:59	Analysis:	Parametric-Multiple Comparison ①	Official Results:	Yes

Batch ID:	16-2471-9213	Test Type:	Survival-Development-Growth	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-013 (2002)~~ ①	Diluent:	Mod-Hard Synthetic Water
Ending Date:	~~03~~ 17 Jun-15 10:35 ①	Species:	~~Pimephales promelas~~ Lithobates pipiens	Brine:	~24 h post hatch ①
Duration:	~~13d~~ 27 21h ①	Source:	Carolina Biological Supply ①	Age:	~~48~~ h

Sample ID:	14-4383-0733	Code:	560F1BCD	Client:	Internal Lab
Sample Date:	~~19 Jun-15~~ ①	Material:	~~Not Applicable~~ NTO ①	Project:	Special Studies
Receive Date:	~~24 Jun-15 17:54~~ ①	Source:	~~Reference Toxicant~~ ①		
Sample Age:	NA	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	23.5%	3070	7775	4886	

Bonferroni Adj t Test ↑ 1-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	0.1089	2.53	4.644	8	1.0000	CDF	Non-Significant Effect
		480	0.4106	2.53	4.926	7	1.0000	CDF	Non-Significant Effect
		1230	-0.4875	2.53	4.926	7	1.0000	CDF	Non-Significant Effect
		3070	-0.1089	2.53	4.644	8	1.0000	CDF	Non-Significant Effect
		7775*	3.677	2.53	5.363	6	0.0037	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	169.0962	33.81923	5	4.008	0.0111	Significant Effect
Error	168.75	8.4375	20			
Total	337.8462		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.7745	4.44	0.5820	Equal Variances
Variances	Levene Equality of Variance	1.528	4.1	0.2259	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9318	0.891	0.0858	Normal Distribution

~~Mean Dry Biomass-mg~~ Summary Snout to Vent Length (mm) ①

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	22.8	19.35	26.25	23	20	26	1.241	12.2%	0.0%
201		5	22.6	17.99	27.21	22	20	29	1.661	16.4%	0.88%
480		4	22	16.97	27.03	21.5	19	26	1.581	14.4%	3.51%
1230		4	23.75	17.74	29.76	23	20	29	1.887	15.9%	-4.17%
3070		5	23	21.04	24.96	23	21	25	0.7071	6.87%	-0.88%
7775		3	15	15	15	15	15	15	0	0.0%	34.2%

~~Mean Dry Biomass~~-mg Detail Snout to Vent Length (mm) ①

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	26	20	23	20	25
201		20	20	22	22	29
480		26	23	19	20	
1230		29	23	23	20	
3070		23	25	21	22	24
7775		15	15	15		

① DDP 10/15/15 E

CETIS Analytical Report

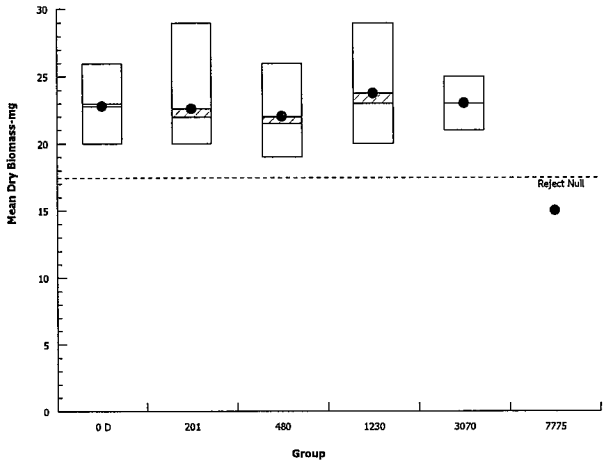
Lithobates pipiens 28-Day ①

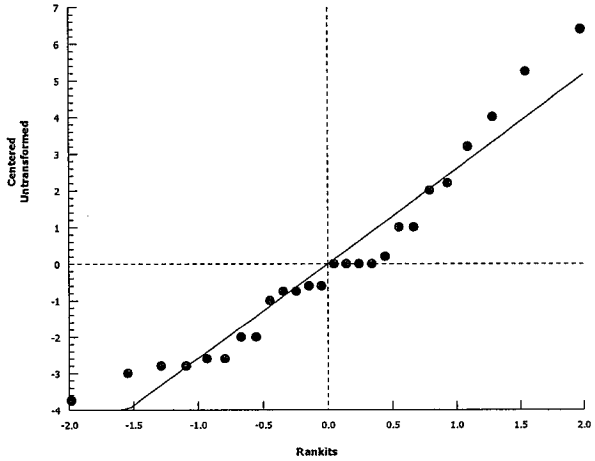
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	15 Oct-15 11:00 (p 2 of 2)
Test Code:	468-010-28svl | 12-3899-7787
	TRE Environmental Strategies, LLC

Analysis ID:	14-9396-3966	Endpoint:	~~Mean Dry Biomass-mg~~ SVL, mm	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 10:59	Analysis:	Parametric-Multiple Comparison ①	Official Results:	Yes

Graphics





① DSP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day (1)

Report Date:	15 Oct-15 11:00 (p 1 of 2)		
Test Code:	468-010-28svl | 12-3899-7787		

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	20-4152-8016	Endpoint:	~~Mean Dry Biomass-mg~~ SVL, mm (1)	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 11:00	Analysis:	Parametric-Multiple Comparison	Official Results:	Yes

Batch ID:	16-2471-9213	Test Type:	Survival-Development-Growth	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-013 (2002)~~ (1)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	~~03~~ 17 Jun-15 10:35 (1)	Species:	~~Pimephales promelas~~ Lithobates pipiens (1)	Brine:	~24 h post hatch
Duration:	~~13d~~ 27 21h (1)	Source:	Carolina Biological Supply	Age:	~~48~~ h (1)

Sample ID:	14-4383-0733	Code:	560F1BCD	Client:	Internal Lab
Sample Date:	~~19 Jun-15~~ (1)	Material:	~~Not Applicable~~ NTO (1)	Project:	Special Studies
Receive Date:	~~24 Jun-15 17:54~~ (1)	Source:	~~Reference Toxicant~~ (1)		
Sample Age:	NA	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C <> T	NA	NA	26.5%	3070	7775	4886	

Bonferroni Adj t Test ↑ 2-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	0.1089	2.85	5.227	8	1.0000	CDF	Non-Significant Effect
		480	0.4106	2.85	5.544	7	1.0000	CDF	Non-Significant Effect
		1230	0.4875	2.85	5.544	7	1.0000	CDF	Non-Significant Effect
		3070	0.1089	2.85	5.227	8	1.0000	CDF	Non-Significant Effect
		7775*	3.677	2.85	6.036	6	0.0075	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	169.0962	33.81923	5	4.008	0.0111	Significant Effect
Error	168.75	8.4375	20			
Total	337.8462		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.7745	4.44	0.5820	Equal Variances
Variances	Levene Equality of Variance	1.528	4.1	0.2259	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9318	0.891	0.0858	Normal Distribution

~~Mean Dry Biomass-mg~~ Summary Snout to Vent Length (mm) (1)

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	22.8	19.35	26.25	23	20	26	1.241	12.2%	0.0%
201		5	22.6	17.99	27.21	22	20	29	1.661	16.4%	0.88%
480		4	22	16.97	27.03	21.5	19	26	1.581	14.4%	3.51%
1230		4	23.75	17.74	29.76	23	20	29	1.887	15.9%	-4.17%
3070		5	23	21.04	24.96	23	21	25	0.7071	6.87%	-0.88%
7775		3	15	15	15	15	15	15	0	0.0%	34.2%

~~Mean Dry Biomass-mg~~ Detail Snout to Vent Length (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	26	20	23	20	25
201		20	20	22	22	29
480		26	23	19	20	
1230		29	23	23	20	
3070		23	25	21	22	24
7775		15	15	15		

(1) DSP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day ①

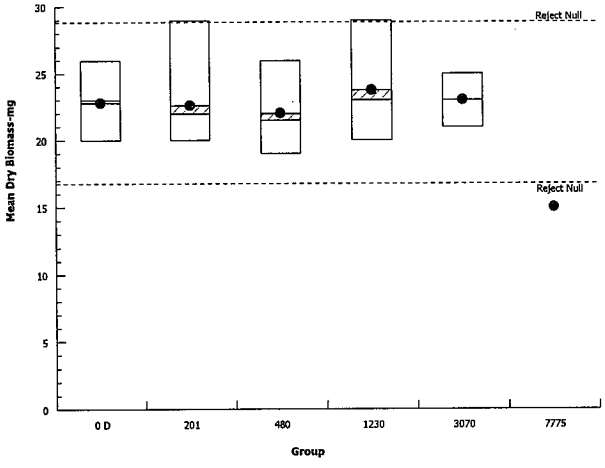
Report Date:	15 Oct-15 11:00 (p 2 of 2)
Test Code:	468-010-28svl | 12-3899-7787

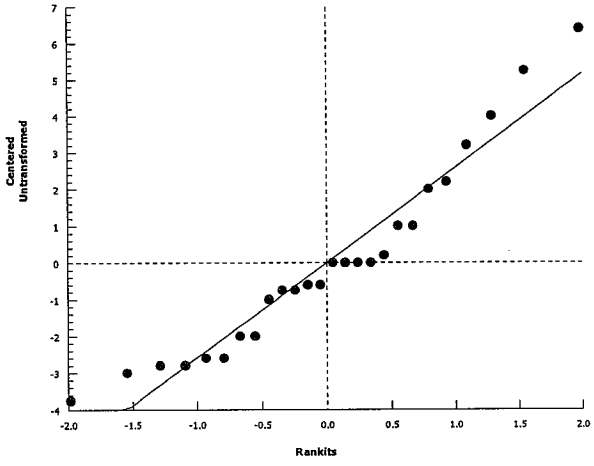
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	20-4152-8016	Endpoint:	~~Mean Dry Biomass-mg~~ SVL, mm ①	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 11:00	Analysis:	Parametric-Multiple Comparison	Official Results:	Yes

Graphics





① DSP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day ①

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	15 Oct-15 11:01 (p 1 of 2)
Test Code:	468-010-28svl | 12-3899-7787
	TRE Environmental Strategies, LLC

Analysis ID:	02-3242-2562	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL(mm) ①	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:01	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	16-2471-9213	**Test Type:**	Survival-Development-Growth	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-013 (2002)~~ ①	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	~~03~~ 17 Jun-15 10:35 ①	**Species:**	~~Pimephales promelas~~ Lithobates pipiens ①	**Brine:**	~24 h post hatch
Duration:	~~13d~~ 27 21h ①	**Source:**	Carolina Biological Supply	**Age:**	~~48~~ h ①

Sample ID:	14-4383-0733	**Code:**	560F1BCD	**Client:**	Internal Lab
Sample Date:	~~19 Jun-15~~ ①	**Material:**	~~Not Applicable~~ NTO ①	**Project:**	Special Studies
Receive Date:	~~24 Jun-15 17:54~~ ①	**Source:**	~~Reference Toxicant~~ ①		
Sample Age:	NA	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	103871	200	Yes	Two-Point Interpolation

Point Estimates

Level		95% LCL	95% UCL
IC5	3515	N/A	3562
IC10	4025	N/A	4132
IC15	4609	2968	4793
IC20	5278	3572	5560
IC25	6044	4300	6449
IC40	>7775	N/A	N/A
IC50	>7775	N/A	N/A

28-Day IC25 on Snout-to-Vent (SVL) Length

~~Mean Dry Biomass-mg~~ Summary Snout to Vent Length (mm) ① Calculated Variate

Group	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	5	22.8	20	26	1.241	2.775	12.2%	0.0%
201		5	22.6	20	29	1.661	3.715	16.4%	0.88%
480		4	22	19	26	1.581	3.162	14.4%	3.51%
1230		4	23.75	20	29	1.887	3.775	15.9%	-4.17%
3070		5	23	21	25	0.7071	1.581	6.87%	-0.88%
7775		3	15	15	15	0	0	0.0%	34.2%

~~Mean Dry Biomass-mg~~ Detail Snout o vent length (mm) ①

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	26	20	23	20	25
201		20	20	22	22	29
480		26	23	19	20	
1230		29	23	23	20	
3070		23	25	21	22	24
7775		15	15	15		

① DAP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day (1)

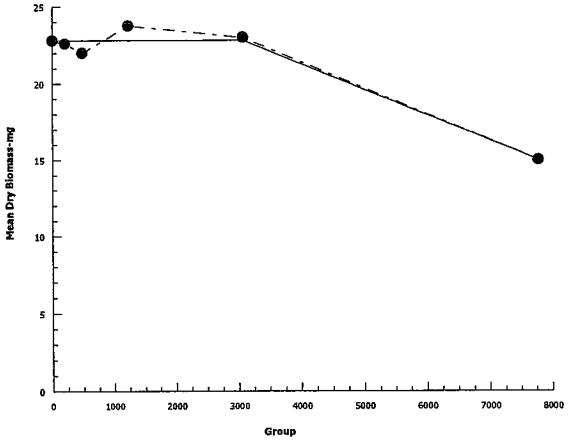
Report Date:	15 Oct-15 11:01 (p 2 of 2)
Test Code:	468-010-28svl | 12-3899-7787

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	02-3242-2562	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL, mm (1)	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:01	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



(1) DSP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day (1)

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — Body Width

Report Date:	15 Oct-15 11:02 (p 1 of 2)		
Test Code:	468-010-28wid | 15-3751-4279		
TRE Environmental Strategies, LLC			

Analysis ID:	03-5889-4448	**Endpoint:**	~~Mean Dry Biomass-mg~~ ~~SVZ~~, mm (1)	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:02	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Batch ID:	16-2471-9213	**Test Type:**	Survival-Development-Growth	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-013 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	17 Jun-15 10:35	**Species:**	~~Pimephales promelas~~ Lithobates pipiens (1)	**Brine:**	~24h post hatch
Duration:	27d 21h	**Source:**	Carolina Biological Supply	**Age:**	~~48~~ h (1)

Sample ID:	21-0485-3082	**Code:**	7D75825A	**Client:**	Internal Lab
Sample Date:	~~19 May-15~~ (1)	**Material:**	~~Not Applicable~~ NTO (1)	**Project:**	Special Studies
Receive Date:	~~25 Jun-15 16:26~~ (1)	**Source:**	~~Reference Toxicant~~ (1)		
Sample Age:	~~38h~~ NA (1)	**Station:**	In House		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	33.4%	3070	7775	4886	

Bonferroni Adj t Test — 1-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	1.613	2.53	3.762	8	0.3063	CDF	Non-Significant Effect
		480	1.267	2.53	3.991	7	0.5493	CDF	Non-Significant Effect
		1230	1.109	2.53	3.991	7	0.7019	CDF	Non-Significant Effect
		3070	1.478	2.53	3.762	8	0.3873	CDF	Non-Significant Effect
		7775*	2.909	2.53	4.344	6	0.0217	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	48.21154	9.642307	5	1.741	0.1712	Non-Significant Effect
Error	110.75	5.5375	20			
Total	158.9615		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.327	4.44	0.3024	Equal Variances
Variances	Levene Equality of Variance	1.981	4.1	0.1256	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9306	0.891	0.0801	Normal Distribution

~~Mean Dry Biomass-mg~~ Summary — Body Width (mm) (1)

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	13	8.272	17.73	12	9	19	1.703	29.3%	0.0%
201		5	10.6	8.025	13.17	10	9	14	0.9274	19.6%	18.5%
480		4	11	7.563	14.44	10.5	9	14	1.08	19.6%	15.4%
1230		4	11.25	7.722	14.78	11	9	14	1.109	19.7%	13.5%
3070		5	10.8	9.181	12.42	10	10	13	0.5831	12.1%	16.9%
7775		3	8	8	8	8	8	8	0	0.0%	38.5%

~~Mean Dry Biomass-mg~~ Detail — Body Width (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	14	9	12	11	19
201		9	9	10	11	14
480		14	11	9	10	
1230		14	10	12	9	
3070		10	13	10	10	11
7775		8	8	8		

(1) DSP 10/15/15 E

CETIS Analytical Report

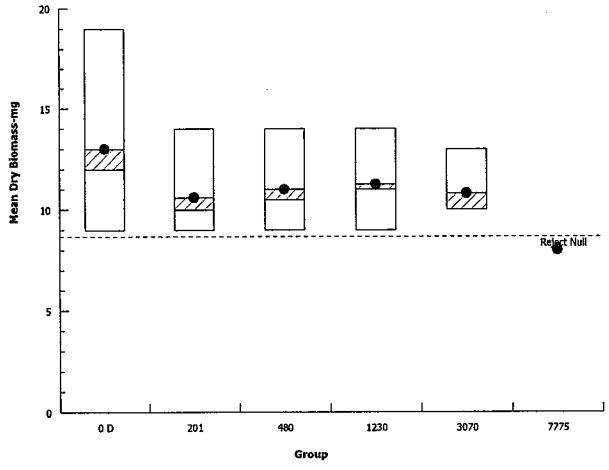
Lithobates pipiens 28-Day ①

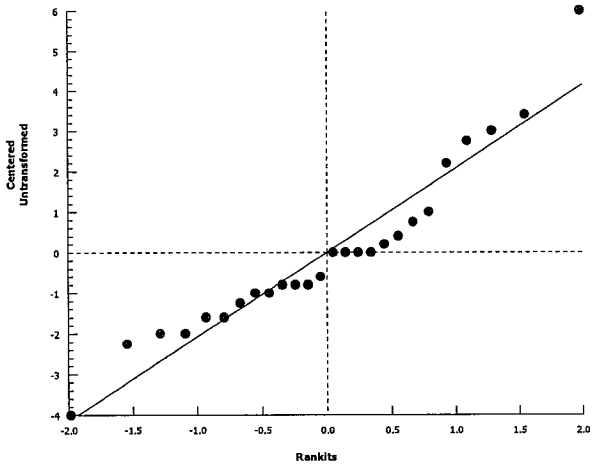
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	15 Oct-15 11:02 (p 2 of 2)
Test Code:	468-010-28wid | 15-3751-4279
TRE Environmental Strategies, LLC	

Analysis ID:	03-5889-4448	**Endpoint:**	~~Mean Dry Biomass-mg~~ Body with, mm ①	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:02	**Analysis:**	Parametric-Multiple Comparison ①	**Official Results:**	Yes

Graphics





①DSP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day (1)

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	15 Oct-15 11:03 (p 1 of 2)
Test Code:	468-010-28wid | 15-3751-4279
	TRE Environmental Strategies, LLC

Analysis ID:	21-2730-0062	**Endpoint:**	~~Mean Dry Biomass-mg~~ Body width-mm	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:02	**Analysis:**	Parametric-Multiple Comparison (1)	**Official Results:**	Yes

Batch ID:	16-2471-9213	**Test Type:**	Survival-Development-Growth	**Analyst:**	Lab Tech
Start Date:	20 May-15 13:30	**Protocol:**	~~EPA/821/R-02-013 (2002)~~ (1)	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	17 Jun-15 10:35	**Species:**	~~Pimephales promelas~~ Lithobates pipiens (1)	**Brine:**	~24 h post-hatch
Duration:	27d 21h	**Source:**	Carolina Biological Supply	**Age:**	1-~~48 h~~ (1)

Sample ID:	21-0485-3082	**Code:**	7D75825A	**Client:**	Internal Lab
Sample Date:	~~19 May-15~~ (1)	**Material:**	~~Not Applicable~~ NTO (1)	**Project:**	Special Studies
Receive Date:	~~25 Jun-15 16:26~~ (1)	**Source:**	~~Reference Toxicant~~ (1)		
Sample Age:	~~38h~~ (1)	**Station:**	In House		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C <> T	NA	NA	37.6%	3070	7775	4886	

Bonferroni Adj t Test ↑ 2-Tailed Test

Control	vs	Group	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	1.613	2.85	4.235	8	0.6125	CDF	Non-Significant Effect
		480	1.267	2.85	4.492	7	1.0000	CDF	Non-Significant Effect
		1230	1.109	2.85	4.492	7	1.0000	CDF	Non-Significant Effect
		3070	1.478	2.85	4.235	8	0.7746	CDF	Non-Significant Effect
		7775*	2.909	2.85	4.89	6	0.0433	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	48.21154	9.642307	5	1.741	0.1712	Non-Significant Effect
Error	110.75	5.5375	20			
Total	158.9615		25			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.327	4.44	0.3024	Equal Variances
Variances	Levene Equality of Variance	1.981	4.1	0.1256	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9306	0.891	0.0801	Normal Distribution

~~Mean Dry Biomass-mg~~ Summary Body width (mm) (1)

Group	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	5	13	8.272	17.73	12	9	19	1.703	29.3%	0.0%
201		5	10.6	8.025	13.17	10	9	14	0.9274	19.6%	18.5%
480		4	11	7.563	14.44	10.5	9	14	1.08	19.6%	15.4%
1230		4	11.25	7.722	14.78	11	9	14	1.109	19.7%	13.5%
3070		5	10.8	9.181	12.42	10	10	13	0.5831	12.1%	16.9%
7775		3	8	8	8	8	8	8	0	0.0%	38.5%

~~Mean Dry Biomass-mg~~ Detail Body width (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	14	9	12	11	19
201		9	9	10	11	14
480		14	11	9	10	
1230		14	10	12	9	
3070		10	13	10	10	11
7775		8	8	8		

(1) DSP 10/15/15 E

CETIS Analytical Report

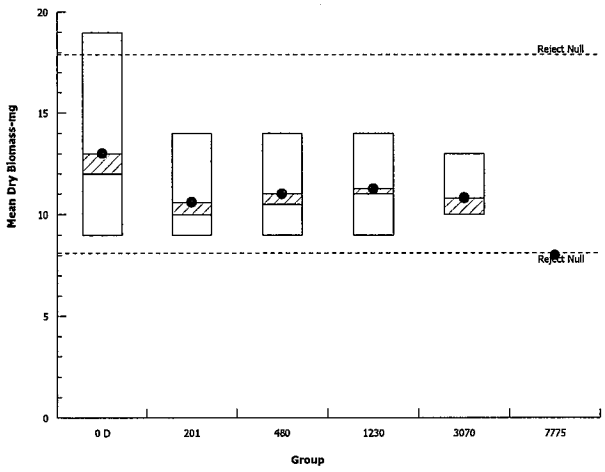
Lithobates pipiens 28-Day (1)

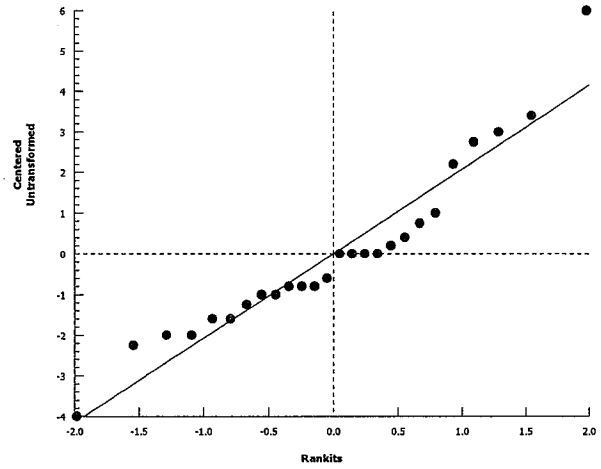
Report Date:	15 Oct-15 11:03 (p 2 of 2)
Test Code:	468-010-28wid | 15-3751-4279

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	21-2730-0062	Endpoint:	~~Mean Dry Biomass-mg~~ Body width, mm	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 11:02	Analysis:	Parametric-Multiple Comparison (1)	Official Results:	Yes

Graphics





(1) DAD 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day (1)

Report Date:	15 Oct-15 11:03 (p 1 of 2)
Test Code:	468-010-28wid | 15-3751-4279

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	04-4211-7522	Endpoint:	~~Mean Dry Biomass-mg~~ Body width (mm)	CETIS Version:	CETISv1.8.7
Analyzed:	15 Oct-15 11:03	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Batch ID:	16-2471-9213	Test Type:	Survival-Development-Growth	Analyst:	Lab Tech
Start Date:	20 May-15 13:30	Protocol:	~~EPA/821/R-02-013 (2002)~~ (1)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	17 Jun-15 10:35	Species:	~~Pimephales promelas~~ Lithobates pipiens (1)	Brine:	~24h post-hatch
Duration:	27d 21h	Source:	Carolina Biological Supply	Age:	~~48~~ h (1)

Sample ID:	21-0485-3082	Code:	7D75825A	Client:	Internal Lab
Sample Date:	~~19 May-15~~ (1)	Material:	~~Not Applicable~~ NTO (1)	Project:	Special Studies
Receive Date:	~~25 Jun-15 16:26~~ (1)	Source:	Reference Toxicant (1)		
Sample Age:	~~38h~~ (1)	Station:	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	916797	200	Yes	Two-Point Interpolation

Point Estimates

Level		95% LCL	95% UCL
IC5	4.382	N/A	5375
IC10	27.97	N/A	6434
IC15	154.9	N/A	7658
IC20	3506	N/A	7443
IC25	4350	N/A	9095
IC40	>7775	N/A	N/A
IC50	>7775	N/A	N/A

28-Day IC25 on Body width

~~Mean Dry Biomass-mg~~ Summary Body width (mm) (1)

		Calculated Variate							
Group	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	5	13	9	19	1.703	3.808	29.3%	0.0%
201		5	10.6	9	14	0.9274	2.074	19.6%	18.5%
480		4	11	9	14	1.08	2.16	19.6%	15.4%
1230		4	11.25	9	14	1.109	2.217	19.7%	13.5%
3070		5	10.8	10	13	0.5831	1.304	12.1%	16.9%
7775		3	8	8	8	0	0	0.0%	38.5%

~~Mean Dry Biomass-mg~~ Detail Body width (mm) (1)

Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	14	9	12	11	19
201		9	9	10	11	14
480		14	11	9	10	
1230		14	10	12	9	
3070		10	13	10	10	11
7775		8	8	8		

(1) DSP 10/15/15 E

CETIS Analytical Report

Lithobates pipiens 28-Day (1)

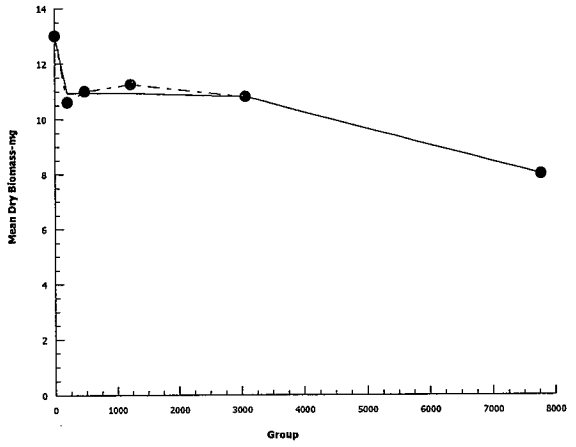
Report Date:	15 Oct-15 11:03 (p 2 of 2)		
Test Code:	468-010-28wid | 15-3751-4279		

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	04-4211-7522	**Endpoint:**	~~Mean Dry Biomass-mg~~ SVL, mm (1)	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Oct-15 11:03	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



(1) DAP 10/15/15 E

US Army Public Health Center
Aberdeen Proving Ground, MD

Study Contract Title: One Generation Amphibian Study
W91ZLK-14-P-0372

APPENDIX D

DEFINITIVE TEST
DATA PACKAGES & STATISTICAL ANALYSES

Prepared by

TRE Environmental Strategies

TRE Environmental Strategies, LLC
Fort Collins, CO

March 2016

TRE Project Number: 15001-468-710

TOXICITY DATA PACKAGE COVER SHEET

QA: AR 12/9/15

Test Type: ~~Acute~~ (1) Chronic

Project Number: 15001-468-010

Test Substance: NTO - pH-adjusted

Species: *Rana pipiens* (Lithobates pipiens) (4)

Dilution Water: Mod Hard

Organism Lot or Batch Number: 15-018

Concurrent Control Water: NA

Age: ~24 h post hatch (24-72 h) Supplier: Carolina Biological

Date and Time Test Began: 5/20/15 @ 1200

Date and Time Test Ended: 7/29/15 @ 0700

Protocol Number:

Investigator(s): DLP/SP/AB

Background Information

Type of Test: Static-Renewal

pH control?: Yes **No**

If yes, give % CO_2: NA

Test Temperature: (1) 22 ± 3°C

Environmental Chamber #: 1

Test Chmbrs: 500-ml cups/beakers & 2.5 gallon aquaria (3)

Photoperiod: 16 h light : 8 h dark

Light Intensity: 100-300 ft-c.

Test Solution Vol.: 250 - 3000 ml

Replicates per Treatment: 4

Length of Test: to metamorphosis (~45-70 d)

Organisms per Replicate: 5

Type of Food and Quantity per Chamber: (2) Grnd Tetrafin - variable

Feeding Frequency: (2) Monday, Wednesday, Friday

Test Substance Characterization Parameters and Frequency:

Food Lots: F10-007 (5/22/15 – 6/24/15) DAP; F15-013 (6/26/15 – 7/27/15) 3/11/16

Hardness: Initiation Alkalinity: Initiation NH_3: Initiation TRC: NA

pH: Init and Term Conductivity: Init and Term

Test Concentrations (Volume:Volume): 0 (Mod Hard), 95, 239, 597, 1492, 3730, 5595 mg/L NTO (Nominal)

Agency Summary Sheet(s)?: NA

0, 95, 239, 597, 1492, 3730, 5595 mg/L

Reference Toxicant Data: Test Dates: NA to NA IC_{25}: NA

Hist. 95% Control Limits: NA to NA Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations:

Note: tadpoles were at Gosner Stages 21-22 at Initiation; left & right external gills apparent

Note: Chambers examined for dead organisms on Saturday & Sunday; dead organisms or unusual conditions recorded. Otherwise, no data are recorded, other than temperature. DAP 6/28/15

Study Director Initials: DLP Date: 5/20/15

(1) DAP 5/20/15 E

(2) Feeding will commence after external gill closure @ Gosner stage 25. See Protocol for amounts. DAP 5/20/15

(3) Plastic cups used for 1st week of test. DAP 10/16/15

(4) Species current name is Lithobates pipiens. DAP 12/9/15

QA Form No. 014
Revision 1
Effective 02/14

TEST SUBSTANCE USAGE LOG

Project Number: 15001-468-010

QA: AR 12/9/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	GLP-066			
Test Substance Collection Date and Time	From: @ NA	From: @	From: @	From: @
	To: @ NA	To: @	To: @	To: @
Sample Type (Grab or Comp)	NA			
Date Test Substance Received	2/16/15			
Dilution Water Number RW# or TRE#, circle one	11644	11658	11668	11675
Concurrent Control Water RW#	NA			
Date(s) Used	5/20/15	5/27/15 (Day 7)	6/3/15 (Day 14)	6/10/15 (Day 21)

Preparation of Test Solutions

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	1500	1500						
1.28%	19.2	1480.8	1500						
3.2%	48	1452	1500						
8%	120	1380	1500						
20%	300	1200	1500						
50%	750	750	1500						
75%	1125	375	1500						
Total	2362.2	8137.8	10500						

Initials / Date	DAP/SP 5/20/15		
Initials / Date		New solutions prepared on Days 7 (5/27), 14 (6/3), 21 (6/10), 28 (6/17), 35 (6/24), 42 (7/1) + 49 (7/8)	
Initials / Date	See Spreadsheet for additional mixing volumes. DAP 5/27/15		
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			

TEST SUBSTANCE USAGE LOG (continued)

Project Number: 15001-468-010

QA: AR 12/9/15

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number				
Test Substance Collection Date and Time	From: @	From: @	From: @	From: @
	To: @	To: @	To: @	To: @
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	11692	11702	11727	
Concurrent Control Water RW#				
Date(s) Used	6/17/15 (Day 28)	6/24/15 (Day 35)	7/8/15 (Day 49)	
		7/1/15 (Day 42)		

Preparation of Test Solutions

Test Substance Conc. (% Effluent)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
MH	0	1500	1500						
1.28%	19.2	1480.8	1500						
3.2%	48	1452	1500						
8%	120	1380	1500						
20%	300	1200	1500						
50%	750	750	1500						
75%	1125	375	1500						
Total	2362.2	8137.8	10500						

Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			

① DSP 12/11/15 E

N. Leopard Frog Lithobates pipiens ③
~~FATHEAD MINNOW (*PIMEPHALES PROMELAS*)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 QA: AR 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
MH	A	5	5	5			5		5	
	B	5	5	5			5		5	
	C	5	5	5			5		5	
	D	5	5	5			5		5	
1.28%	A	5	5	5			5		5	
	B	5	5	5			5		5	
	C	5	5	5			5		5	
	D	5	5	5			5		5	
3.2%	A	5	5	5			5		5	
	B	5	5	5			5		5	
	C	5	5	5			5		5	
	D	5	5	5			5		5	
8%	A	5	5	5			5		5	
	B	5	5	5			5		5	
	C	5	5	5			5		5	
	D	5	5	5			5		4	
20.0%	A	5	4	4	~~4~~ ①		4		4	
	B	5	5	5			5		5	
	C	5	5	5			5		5	
	D	5	5	5			5		4	
50.0%	A	5	5	5			5		~~3~~ 4 ②	
	B	5	5	5			5		~~2~~ 8 ②	
	C	5	5	5			5		2	
	D	5	5	5			5		~~4~~ 8 ②	
75.0%	A	5	1	0			–			Survivors on Day 1 very
	B	5	2	0			–			lethargic
	C	5	3	0			–			↓
	D	5	3	0			–			↓
Date:		5/20/15	5/21/15	5/22/15			5/25/15		5/27/15	
Time:		1200	1425	1450			1100		1400	
Initials:		DSP/SP	DSP	DSP			DSP		DSP	

① DSP 5/25/15 WP ② DSP 5/27/15 E ③ DSP 6/29/15 E ④ DSP 12/11/15 E

N. Leopard Frog Lithobates pipiens ③
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 QA: DRE 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Remarks
MH	A	5	5			5	5	5	5	
	B	5	5			5	5	5	5	
	C	5	5			5	5	5	5	
	D	5	5			5	5	5	5	
1.28%	A	5	5			5	5	② ~~5~~ 4	4	DITE
	B	5	5			5	5	5	5	
	C	5	5			5	5	5	5	
	D	5	5			5	5	5	5	
3.2%	A	5	5			5	5	5	5	
	B	5	5			5	5	5	5	
	C	5	5			5	5	5	5	
	D	5	5			5	5	5	5	
8%	A	5	5			5	5	5	5	
	B	5	5			5	5	5	5	
	C	5	5			5	5	5	5	
	D	4	4			4	4	② ~~5~~ 4	4	
20.0%	A	4	4			4	4	4	4	
	B	5	5			5	5	4	4	
	C	5	5			5	5	5	5	
	D	4	4			4	4	4	4	
50.0%	A	2	2 ~~5~~ ①			2	2	2	2	
	B	2	2			2	2	2	2	
	C	1	1			1	1	1	1	
	D	2	2			2	2	2	2	
75.0%	A									
	B									
	C									
	D									
Date:		5/28/15	5/29/15			6/1/15	6/2/15	6/3/15	6/4/15	
Time:		1600	1105			1135	1548	1045	1527	
Initials:		DAP	DAP			DAP	DAP	AM	DAP	

① DAP 5/29/15 E ② AB 6/3/15 E ③ DAP 6/28/15 E ④ DAP 12/1/15 E

N. Leopard Frog Lithobates pipiens ①
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 QA: AR 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms								
		Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Remarks
MH	A	5			5	5	5	5	5	
	B	5			5	5	5	5	5	
	C	5			5	5	5	5	5	
	D	5			4[a]	4	4	4	4	a; NF
1.28%	A	4			4	4	4	4	4	
	B	5			5	5	5	5	5	
	C	5			5	5	5	5	5	
	D	5			5	5	5[b]	5	5	
3.2%	A	5			5	5	5	5	5	
	B	5			5	5	5	5	5	
	C	5			5	5	5	5	5	
	D	5			5	5	5	5	5	
8%	A	5			5	5	5	5	5	
	B	5			5	5	5	5	5	
	C	5			5	5	5	5	5	
	D	4			4	4[a]	4	4	4	
20.0%	A	4			4	4	4	4	4	
	B	4			4	4	4	4	4	
	C	5			5	5	5	5	5	
	D	4			4	4	4	4	4	
50.0%	A	2			2	2	2	2	2	
	B	2			1	1	1	1	1	
	C	1			1	1	1	1	1	
	D	2			2	2	2	2	2	
75.0%	A									
	B									
	C									
	D									
Date:		6/5/15			6/8/15	6/9/15	6/10/15	6/11/15	6/12/15	
Time:		1520			1145	1645	1100	1717	1420	
Initials:		DAP			DAP	DAP	MB	DAP	MB	

a; Tadpole is smaller, has a tail turned 90° & moves in circles; 2nd tadpole also has turned tail & moving in circles, but is large as remaining 2 tadpoles. DAP 6/9/15
b: Tadpole significantly smaller 6/10/15 MB ① DAP 6/29/15 E ② DAP 12/11/15 E

N. Leopard Frog Lithobates pipiens (4)
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 QA: AR 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Remarks
MH	A			5	5	5	5	5	~~5~~	(3)
	B			5	5	5	5	5	~~5~~	
	C			5	5	5	5	5	~~5~~	
	D			4	4	4	4	4	~~4~~	
1.28%	A			4	4	4	4	4	~~4~~	
	B			5	5	5	5	5	~~5~~	
	C			5	5	5	5	5	~~5~~	
	D			5	5	5	5	5	~~5~~	
3.2%	A			5	5	5	5	5	~~5~~	
	B			5	5	5	5	5	~~5~~	
	C			5	5	5	5	5	~~5~~	
	D			5	5	5	5	5	~~5~~	
8%	A			5	5	5	5	5	~~5~~	
	B			5	5	5	5	5	~~5~~	
	C			5	5	5	5	5	~~5~~	
	D			4	4	4	4	4	~~4~~	
20.0%	A			4	4	4	4	4	~~4~~	(3)
	B			4	4	4	4	4	~~3~~	~~Dead tadpole (Day 31) shows swelling/hemorrhaging near base of tail~~ (photo taken)
	C			5	5	5	5	5	~~5~~	(3)
	D			4	4	4	4	4	~~4~~	
50.0%	A			2	2	~~12~~ 2 a	1	1	+	
	B			1	1	1	1	1	+	
	C			1	1	1	1	1	+	
	D			2	2	2	2	2	2	(3)
75.0%	A									
	B									
	C									
	D			(1)						
Date:				6/15/15	6/16/15	6/17/15	6/18/15	6/19/15	~~6/22/15~~	
Time:				1130	1110	1035	1455	1430	~~1450~~	
Initials:				DAP	DAP	M	DAP	DAP	~~DAP~~	

(1) DAP 6/15/15 WP
(2) DAS 6/16/15 E
(3) DAP 6/22 WP - See next page for Day 33 data
a - one organism floating ventral side up, dead @ removal time 1550. Initially described and bleached
(4) DAP 6/29/15 E
(5) DAP 12/17/15 E

N. Leopard Frog Lithobates pipiens ①
~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 QA: AR 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms								
		Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Remarks
MH	A		5	5	5	5	5			
	B		5	5	5	5	5			
	C		5	5	5	5	5			
	D		4	4	4	4	4			
1.28%	A		4	4	4	4	4			
	B		5	5	5	5	4			
	C		5	5	5	5	5			
	D		5	5	5	5	5			
3.2%	A		5	5	5	5	5			
	B		5	5	5	5	5			
	C		5	5	5	5	4			
	D		5	5	5	5	5			
8%	A		5	5	5	5	5			
	B		5	5	5	5	5			
	C		5	5	5	5	5			
	D		4	4	4	4	3*			*Dead tadpole on day 37 had severely twisted spine was smaller than others DAP
20.0%	A		4	4	4	4	4			
	B		3[a]	3	3	3	3			
	C		5	5	5	5	5			
	D		4	4	4	4	4			
50.0%	A		1	1	1	1	1			
	B		1	1	1	1	1			
	C		1	1	1	1	1			
	D		2	2	2	2	2			
75.0%	A									
	B									
	C									
	D									
Date:			6/22/15	6/23/15	6/24/15	6/25/15	6/26/15			
Time:			1450	1700	1100	1000	1100			
Initials:			DAP	DAP	[illegible]	DAP	DAP			

[a] Dead organism (Day 33) in 20% Rep B shows swollen + ~~red~~ red (hemoraging) color at base of tail (~~picture taken~~ picture not taken). DAP 6/22/15

① DAP 6/29/15 E ② DAP 12/11/15 E

N. Leopard Frog Lithobates pipiens
~~FATHEAD MINNOW (*PIMEPHALES PROMELAS*)~~ ①

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010

QA: AR 10/25/15

%Conc.	Test Replicate	Day 40	Day 41	Day 42	Day 43	Day 44	Day 45 ④	Day 46	Day 47	Remarks
		Number of Surviving Organisms								
MH	A	5[a]	5	5	5	5[b]	4/1/5	4[b]/1/5	3/2/5	~~5~~ ④ Beginning on
	B	5	5	5[c]	5[d]	5[b]	3/2/5	3[b]/2/5	2[b]/3/5	Day 45, Survival
	C	5	5[b]	5	5	5	4/1/5	4/1/5	4/1/5	noted as 3
	D	4[a]	4[b]	4[b] ③	4	4[c]	2[d]/2/4	0/4/4	0/4/4	numbers:
1.28%	A	4	4	4	4	4	4/0/4	4/0/4	4[d]/0/4	# in orig chamber
	B	4	4	4	4	4	4[b]/0/4	3/1/4	3/1/4	# Transferred
	C	5	~~4~~ 5[c] ⑤	4[b]	4	4	3/1/4	3/1/4	3[b]/1/4	# Alive Total,
	D	5	5	5	5	5	5/0/5	5[b]/0/5	5/0/5	such as:
3.2%	A	5	5	5	5	5[b]	5/0/5	4/1/5	4/1/5	"3/2/5" means
	B	5	5	5	5	5	5/0/5	4/1/5	4[b]/1/5	3 in Tank
	C	4	4	4[b]	4	4	3/1/4	3/1/4	3/1/4	2 Transferred
	D	5[a]	5	5	5	5	4/1/5	4[d]/1/5	2/~~3~~/1/5 ⑥	5 Alive total
8%	A	5	5[b]	5	5	5	4/1/5	4[c]/1/5	4[b,c]/1/5	at the time
	B	5	5	5	5	5	5/0/5	5/0/5	5/0/5	of transfer
	C	5	5	5[b]	5	5	4/1/5	4/1/5	4/1/5	Note: Animals
	D	3	3	3	3[b]	3	3/0/3	2/1/3	2/1/3	alive at the
20.0%	A	4	4	4	4	4	4/0/4	4/0/4	4/0/4	time of transfer
	B	3	3	3	3	3	3/0/3 ⑥	3/0/3	3/0/3	~~are~~ and are
	C	5	5	5	5	5	5[b]/0/5	5/0/5	4/1/5	~~alive~~ ⑤ euthanized
	D	4	4	4	4	4	4/0/4	4/0/4	4/0/4	after completion
50.0%	A	1	1	1	1	1	0/0/0	—	—	of metamorphosis
	B	1	1	1	1	1	1/0/1	1/0/1	1/0/1	are considered
	C	1	1	1	1	1	1/0/1	1/0/1	1/0/1	alive on these
	D	2	2	1	1	1	1/0/1	1/0/1	1/0/1	data sheets. DAP 7/4/15
75.0%	A									
	B									
	C									
	D									
	Date:	6/29/15	6/30/15	7/1/15	7/2/15	7/3/15	7/4/15	7/5/15	7/6/15	⑦ DAP 12/11/15 E
	Time:	1415	1140	1160	1215	1055	1500	1147	1200	
	Initials:	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP	

a) in each rep with front limbs. 6/29/15 DAP

① DAP 6/29/15 E

② DAP 6/30/15 E (Died after Init. Count)

③ DAP 7/1/15 E

④ DAP 7/4/15 E

b ~~and~~ 1 tadpole w front limbs. 6/30/15 DAP

⑤ DAP 7/1/15 E

c one front limb (Right) emerged in 1 animal

d Two (2) tadpoles with front limbs DAP 7/2/15

⑥ DAP 7/7/15 E

④ Northern Leopard Frog (Lithobates pipiens)

~~FATHEAD MINNOW (*PIMEPHALES PROMELAS*)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010 QA: MR 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms								Remarks
		Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54	Day 55	
MH	A	3/2/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	
	B	2/3/5	1/4/5	1/4/5	1c/4/5	1/4/5	0/5/5	0/5/5	0/5/5	
	C	4c/1/5	4/1/5	3/2/5	3c/2/5	3/2/5	2/3/5	2/3/5	2/3/5	
	D	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	
1.28%	A	4/0/4	2/2/4	2/2/4	2/2/4	2/2/4	2/2/4	2/2/4	2/2/4	
	B	3/1/4	3/1/4	3/1/4	3c/1/4	3/1/4	2c/2/4	1/3/4	1/3/4	
	C	3/1/4	2/2/4	2/2/4	2/2/4	2/2/4	2/2/4	2/1/3	2/1/3	a Transferred Tadpole was
	D	5c/0/5	4c/1/5	4/1/5	4/1/5	4c/1/5	3c/2/5	2/3/5	2/3/5	Dead in amagonca chamber DSP
3.2%	A	4/1/5	4/1/5	4c/1/5	4/1/5	3/2/5	3c/2/5	3/2/5	2/3/5	
	B	4/1/5	3/2/5	3/2/5	3c/2/5	2/2/5	2/3/5	2/3/5	2/3/5	
	C	3/1/4	3c/1/4	3/1/4	3c/1/4	2/2/4	2/2/4	2/2/4	2c/2/4	
	D	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2c/3/5	2/3/5	1/4/5	
8%	A	4/1/5	2/3/5	2/3/5	2c/3/5	2/3/5	1/4/5	1c/4/5	1/4/5	
	B	5b/0/5	5/0/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	
	C	4/1/5	4c/1/5	4/1/5	4c/1/5	3/2/5	3/2/5	3/2/5	3/2/5	
	D	2c/1/3	2/1/3	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	
20.0%	A	4/0/4	4/0/4	4/0/4	4c/0/4	4/0/4	3/1/4	3/1/4	3/1/4	
	B	3/0/3	3/0/3	3/0/3	3/0/3	3/0/3	3/0/3	3/0/3	3/0/3	
	C	4/1/5	4/1/5	4/1/5	4/1/5	4c/1/5	3/2/5	3/2/5	3/2/5	
	D	4/0/4	4/0/4	4/0/4	4c/0/4 ②	3c/0/4 ~~5/0/5~~	4/0/4	~~3/1/4~~ ③	4/0/4	
50.0%	A									4c/0/4 for 7/13/15 DSP
	B	1/0/1	1/0/1	1/0/1	1/0/1	0/0/0				
	C	0/0/0								
	D	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	
75.0%	A									
	B									
	C									
	D									
Date:		7/7/15	7/8/15	7/9/15	7/10/15	7/11/15	7/12/15	7/13/15	7/14/15	
Time:		1335	1200	1315	1030	0735	1315	1125	1110	
Initials:		DSP	DSP	DSP	DSP	DSP	DSP	DSP	DSP	

a One (Right) Front limb erupted 7/7/15 DSP

b Three w Front limbs DSP 7/7/15

c Both Front limbs erupted DSP 7/7/15

① DSP 7/10/15 E ② DSP 7/11/15 E

③ DSP 7/13/15 E ④ DSP 10/8/15 E ⑤ DSP 12/14/15 E

③ Northern Leopard Frog (Lithobates pipiens)

~~FATHEAD MINNOW (PIMEPHALES PROMELAS)~~

CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010

QA: AR 10/25/15

%Conc.	Test Replicate	Number of Surviving Organisms								Remarks
		Day 56	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	
MH	A	[a]2/3/5	2/3/5	1/4/5	1/[a]3/4	1/3/4	1/3/4	1/3/4	1/3/4	[a]Tadpole transformed 7/17/15 Died DAP
	B	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5	Dead Tadpole, had
	C	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	twisted spine.
	D	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	
1.28%	A	2[b]/2/4	2/2/4	2[b]/2/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	
	B	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	
	C	2/1/3	2/1/3	2/1/3	2/1/3	2/1/3	2/1/3	2/1/3	2[b]/1/3	
	D	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	
3.2%	A	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	
	B	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	
	C	2/2/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	
	D	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5	1/~~3~~4/5	1/~~3~~4/5	
8%	A	0/5/5	0/~~4~~4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4 ④	0/4/4 ④	[a]Tadpole Transformed OR 7/15/15 Died DAP
	B	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2[b]/3/5	2/3/5	1/4/5	
	C	3[b]/2/5	3/2/5	2/3/5	2/3/5	2/3/5	2[b]/3/5	2[b]/3/5	0/5/5	
	D	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	
20.0%	A	3[b]/1/4	3/1/4	2/2/4	2/2/4	2/2/4	2/2/4	2/2/4	2/2/4	
	B	3/0/3	3/0/3	3[b]/0/3	3/0/3	2/1/3	2/1/3	2/1/3	2/1/3	
	C	3[b]/2/5	3/2/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2[b]/3/5	
	D	3/1/4	3/1/4	3/1/4	3/1/4	~~2/3/5~~ ① 0/3/1/4	3[b]/1/4	2/2/4	2/2/4	
50.0%	A									
	B									
	C									
	D	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	1/0/1	0*	*Last 50% Tadpole died
75.0%	A									
	B									
	C									
	D									
	Date:	7/15/15	7/16/15	7/17/15	7/18/15	7/19/15	7/20/15	7/21/15	7/22/15	
	Time:	1200	0815	1055	1530	0850	0912	0955	1045	
	Initials:	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP	

[a] One Front limb Emerged (Left limb) 7/15/15 DAP [b] Both Front limbs emerged 7/15/15 DAP

① DAP 7/19/15 E [c] One Front limb (Right) Emerged 7/20/15 DAP

② DAP 7/28/15 E - Late Entry ③ DAP 10/15/15 E ④ DAP 12/11/15 E

④ Northern Leopard Frog (Lithobates pipiens)

~~FATHEAD MINNOW (*PIMEPHALES PROMELAS*)~~
CHRONIC BIOLOGICAL DATA

Project Number: 15001-468-010

QA: AR 10/25/15

%Conc.	Test Replicate	Day 64	Day 65	Day 66	Day 67	Day 68	Day 69	Day 70	Day 71	Remarks
		Number of Surviving Organisms								
MH	A	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4	1/3/4		
	B	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5	0/5/5		
	C	2b/3/5	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5		
	D	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4		
1.28%	A	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4		
	B	1/3/4	1/3/4	1b/3/4	1/3/4	0/4/4	0/4/4	0/~~4~~/4 ④		Δ Transferred tadpole (7/27) dead in emerg. chamber.
	C	2/1/3	1/2/3	1/2/3	1/2/3	1/2/3	1/2/3	0/3/3		
	D	2/3/5	2b/3/5	2/3/5	2/3/5	1/4/5	1/4/5	1/4/5		
3.2%	A	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5		
	B	2/3/5	2/3/5	2/3/5	2/3/5	2/3/5	2b/3/5	1/4/5		
	C	1b/3/4	0/4/4	0/4/4	0/4/4	0/4/4	0/~~4~~/4 ③	0/3/3		* Tadpole transf. on 7/24 died in emerg chamber
	D ② →	1/~~3~~/4 5	1/~~3~~/4 5	1/~~3~~/4 5	1/~~3~~/4 5	1/~~3~~/4 5	1/~~3~~/4 5	1/4/5		
8%	A	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4	0/4/4		
	B	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5	1/4/5		
	C ② →	0/~~4~~/4 5	0/~~4~~/4 5	0/~~4~~/4 5	0/~~4~~/4 5	0/~~4~~/4 5	0/~~4~~/4 5	0/5/5		
	D	1/2/3	1/2/3	1/2/3	1/2/3	0/2/2	0/2/2	0/2/2		
20.0%	A	2a/2/4	1/3/4	1/3/4	1/3/4	1/3/4	1/2*/3	1/2/3		* Tadpole transferred on 7/24/15 dead in emerg. chamber.
	B	2/1/3	2/1/3	2/1/3	2a/1/3	0/3/3	0/3/3	0/3/3		
	C	2/3/5	1/4/5	0/3/3	0/3/3	0/3/3	0/3/3	0/3/3		Δ Tadpole transferred on 7/24/15 is Dead in emergence chamber + 1 dead tadpole in exposure chamber. So only 3 remaining survived thru metamorphosis. DAP 7/25/15
	D	2/2/4	2/2/4	2/2/4	2/2/4	1/2/3	1c/2/3	0/3/3		
50.0%	A	—	—	—	—	—	—	—	—	
	B	—	—	—	—	—	—	—	—	
	C	—	—	—	—	—	—	—	—	
	D	—	—	—	—	—	—	—	—	
75.0%	A	—	—	—	—	—	—	—	—	
	B	—	—	—	—	—	—	—	—	
	C	—	—	—	—	—	—	—	—	
	D	—	—	—	—	—	—	—	—	
	Date:	7/23/15	7/24/15	7/25/15	7/26/15	7/27/15	7/28/15	7/29/15		
	Time:	1515	1010	1445	1000	1310	1430	0700		
	Initials:	DAP	DAP	DAP	DAP	DAP	DAP	DAP		

a One ~~two~~ front limb (Right) erupted. DAP 7/23/15 b Both front limbs erupted DAP 7/23/15
c one front limb (left) erupted. DAP 7/24/15 ① DAP 7/28/15 E ② DAP 7/28/15 E
③ DAP 7/29/15 E ④ DAP 10/19/15 E ⑤ DAP 12/11/15 E

Late Entry

Initial Chemistry Data – Each Replicate

Project Number: 15001-468-010

QA: AR 12/9/15

	Day: 0 (Initiation)				Day:			
	Replicate				Replicate			
Conc.: 0	A	B	C	D	A	B	C	D
pH	8.2	8.2	8.2	8.2				
DO (mg/L)	6.7	6.7	6.7	6.7				
Temp. (°C)	20.5	20.3	20.4	20.2				
Conc.: 1.28%								
pH	8.2	8.2	8.2	8.2				
DO (mg/L)	6.6	6.6	6.6	6.6				
Temp. (°C)	21.0	20.4	20.5	20.2				
Conc.: 3.2%								
pH	8.1	8.1	8.1	8.1				
DO (mg/L)	6.5	6.5	6.6	6.6				
Temp. (°C)	20.4	20.5	20.2	20.4				
Conc.: 8%								
pH	8.1	8.1	8.1	8.1				
DO (mg/L)	6.6	6.6	6.6	6.6				
Temp. (°C)	20.5	21.1	21.3	20.9				
Conc.: 20%								
pH	8.0	8.0	8.0	8.0				
DO (mg/L)	6.4	6.4	6.5	6.6				
Temp. (°C)	20.3	20.3	20.4	20.4				
Conc.: 50%								
pH	7.9	7.9	7.9	7.9				
DO (mg/L)	6.7	6.7	6.6	6.6				
Temp. (°C)	20.3	20.3	20.4	20.7				
Conc.: 75%								
pH	7.6	7.6	7.6	7.7				
DO (mg/L)	6.7	6.6	6.6	6.6				
Temp. (°C)	20.4	20.6	20.4	20.5				
Date:	5/20/15	→	→	→				
Time:	1230	→	→	→				
Initials:	DJP	→	→	→				

CHRONIC CHEMICAL DATA ~~(INITIAL)~~ ALL ③

Project Number: 15001-468-010 QA: DR 12/19/15

Test Species: ~~Rana~~ Lithobates ③ pipiens

%	Day 0	Day 1	Day 2	Day 5	Day 7	Day 7	② Day ~~8~~ 9	Day 12	Meter #	Remarks
	New	old	Old	Old	old	New	old	old		
Conc.: MH			RepA ↓	RepB ↓	RepC ↓	Comp.	RepD ↓	RepA ↓	All Conc.	
pH	8.3		7.8	7.9	8.2	8.1	8.1	8.1	FM26	
D.O. (mg/L)	6.5	5.8	6.3	6.6	5.7	6.4	6.2	5.7	6	
Temp. (°C)	22.3		23.2	23.8	24.2	22.1	23.7	25.1	L-25	
Cond. (µS/cm)	324		441	512	620	303	541△	343	16	△ Double checked
Hard. (mg/L)	90					88			TITR	w Meter 15. DAP
Alk. (mg/L)	61					58			TITR	
TRC (mg/L)	0.02 5/19					0.02 5/27			22	
NH_3 (mg/L)	<1.0 5/19					<1.0 5/27			HA#1	
Conc.: 1.28%										
pH	8.2		7.8	8.0	8.1	8.1	8.2	8.1		
D.O. (mg/L)	6.7		5.8	6.5	5.3	6.2	6.2	5.7		
Temp. (°C)	21.5	①	~~24~~ 23.7	23.7	24.4	22.9	23.1	24.4		
Cond. (µS/cm)	406		429	505	597	419	430	423		
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 3.2%										
pH	8.2		7.8	8.0	8.1	8.1	8.2	8.1		
D.O. (mg/L)	6.7		5.8	6.6	5.3	6.4	6.2	5.8		
Temp. (°C)	21.1		23.9	23.7	24.4	23.0	22.8	24.2		
Cond. (µS/cm)	549		573	606	690	563	550	563		
Conc.: 8%										
pH	8.2		7.7	8.0	8.0	8.0	8.2	8.1		
D.O. (mg/L)	6.6		5.8	6.5	5.3	6.6	6.2	5.6		
Temp. (°C)	21.2		23.9	23.7	24.3	22.9	23.3	24.6		
Cond. (µS/cm)	893		918	918	1159	887	910	957		
Date:	5/20/15	5/21/15	5/22/15	5/25/15	5/27/15	5/27/15	5/29/15	6/1/15		
Time:	1115	1800	1500	1105	1445	1730	1130	1120		
Initials:	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25~~C ~22°C ③ prior to making the dilution series. ③ ~~The temperature of resulting effluent dilution is assumed to also be 25C.~~

① DAP 5/22/15 E ② DAP 5/29/15 E ③ ~~Lithoba~~ DAP 10/19/15 E ④ DAP 12/11/15 E

ALL (6)

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

QA: AR 12/9/15

Project Number: 15001-468-010

Test Species: ~~*Rana*~~ Lithobates (6) *pipiens*

%	Day 0 (New)	Day 1 (old)	Day 2 (old)	Day 5 (old)	Day 7 (old)	Day 7 (New)	Day ~~8~~ 9 (4) (old)	Day 12 (old)	Meter #	Remarks
Conc.: 20.0%									All Conc.	
pH	8.1		7.8	8.0	8.0	8.0	8.2	8.1		
D.O. (mg/L)	6.5		5.7	6.3	5.3	6.5	6.2	5.6		
Temp. (°C)	21.2		23.7	23.8	24.5	22.9	23.1	25.0		
Cond. (µS/cm)	1763		1853	~~18~~ 1915 (2)	2229	1739	1785	~~18~~ 1925 (5)		
Conc.: 50%										
pH	7.9		7.7	7.9	8.0	7.9	8.1	8.0		
D.O. (mg/L)	6.4	6.0	5.7	6.4	5.3	6.5	6.2	5.7		
Temp. (°C)	21.3		23.7	23.6	23.4	22.9	23.5	24.2		
Cond. (µS/cm)	3790		3930	4150	5480	3770	3760	4050		
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 75%										
pH	7.8		7.7							
D.O. (mg/L)	6.4		6.3							
Temp. (°C)	21.4		23.7	~~7~~ (5)						
Cond. (µS/cm)	5560		5560							
Hard. (mg/L) (1)										
Alk. (mg/L)	425									
TRC (mg/L)										
NH_3 (mg/L)										
Date:	5/20/15	5/21/15	5/22/15	5/25/15	5/27/15	5/27/15	5/29/15	6/1/15		
Time:	1115	1800	1500	1105	1445	1730	1130	1120		
Initials:	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25~~ 22C (8) prior to making the dilution series. ~~The temperature of resulting effluent dilution is assumed to also be 25C.~~ (6)

(7) DAP 12/1/15 E

(1) Couldn't measure hardness w titrametric method due to extreme color change with addition of ISA & Hardness Indicator DAP 5/20/15

(2) DAP 5/25/15 E (3) DAP 5/27/15 E (4) DAP 5/29/15 E (5) DAP 6/1/15 E (6) DAP 10/5/15 E

ALL ③

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

QA: AR 12/9/15

Project Number: 15001-468-010

Test Species: ~~*Rana pipiens*~~ Lithobates ②

%	Day 1-4 (Old)	Day 14 (New)	Day 16 (Old)	Day 19 (Old)	Day 21 (Old)	Day 21 (New)	Day 23 (Old)	Day 26 (Old)	Meter #	Remarks
Conc.: MH	Rep B ↓	Comp.	Rep C ↓	Rep D ↓	Rep A ↓	Comp.	Rep B ↓	Rep C ↓	All Conc.	
pH	8.1	8.4	8.2	8.0	8.2	8.3	8.1	8.1	Fm 26	
D.O. (mg/L)	6.0	6.4	5.6	5.5	5.9	6.6	5.8	5.8	6	
Temp. (°C)	24.6	23.6	23.2	24.2	24.3	24.3	24.4	20.4	L-25	
Cond. (µS/cm)	322	294	358	430	390	301	324	438	16	
Hard. (mg/L)		86				88			TITR	
Alk. (mg/L)		57				61			TITR	
TRC (mg/L)		<0.02 6/3				<0.02 6/8			22	
NH_3 (mg/L)		<1.0 6/3				<1.0 6/8			HA#1	
Conc.: 1.28%										
pH	8.0	8.5	8.2	8.0	8.1	8.3	8.0	8.1		
D.O. (mg/L)	5.8	6.2	5.4	5.5	6.0	6.5	5.6	5.8		
Temp. (°C)	25.3	23.7	23.7	23.4	23.9	24.2	25.0	20.4		
Cond. (µS/cm)	440	396	466	495	487	405	438	500		
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Conc.: 3.2%										
pH	8.0	8.5	8.0	8.0	8.1	8.3	8.0	8.1		
D.O. (mg/L)	~~5.9~~ ~~5.7~~ ①	6.3	5.4	5.7	5.9	6.5	5.6	5.8		
Temp. (°C)	24.5	24.1	23.4	23.2	24.0	24.3	24.3	20.3		
Cond. (µS/cm)	574	548	603	672	679	586	641	645		
Conc.: 8%										
pH	8.0	8.4	8.0	8.0	8.0	8.2	8.1	8.1		
D.O. (mg/L)	6.1	6.2	5.7	5.8	5.8	6.5	5.7	5.8		
Temp. (°C)	23.6	24.1	23.7	24.1	24.1	24.3	23.8	20.2		
Cond. (µS/cm)	1064	961	1034	1088	1083	1003	1066	1077		
Date:	6/3/15	6/3/15	6/5/15	6/8/15	6/10/15	6/10/15	6/12/15	6/15/15		
Time:	1100	1355	1500	1200	1050	1410	1425	1207		
Initials:	AB	AB	DAP	DAP	AB	AB	AB	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25~~ 22°C ② °C prior to making the dilution series. ② T~~he temperature of resulting effluent dilution is assumed to also be 25°C.~~

① AB 6/3/15 WP ② DAP 10/19/15 E ③ DAP 12/11/15 E

CHRONIC CHEMICAL DATA ~~(INITIAL)~~ ALL ③

QA: AR 12/9/15

Project Number: 15001-468-010

Test Species: ~~Rana pipiens~~ Lithobates ③

%	Day 14 Old	Day 14 New	Day 16 Old	Day 19 Old	Day 21 Old	Day 21 New	Day 23 Old	Day 26 Old	Meter #	Remarks
Conc.: 20.0%									All Conc.	
pH	8.0	8.2	8.1	8.1	8.0	8.1	8.2	8.1		
D.O. (mg/L)	6.1	6.2	6.0	5.8	5.8	6.5	5.9	5.6		
Temp. (°C)	24.4	23.8	23.3	23.4	24.1	24.4	23.7	20.4		
Cond. (µS/cm)	~~1943~~ 1911 ①	1905	1981	2141	2069	1951	2058	2119		
Conc.: 50%										
pH	7.8	7.7	8.0	8.0	8.0	7.8	8.0	8.0		
D.O. (mg/L)	5.8	6.2	6.9	5.8	6.0	6.5	6.0	5.8		
Temp. (°C)	24.9	24.2	23.7	23.8	23.6	24.3	24.5	20.4		
Cond. (µS/cm)	4260	4100	4050	4570	4350	4150	4350	4440		
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 75%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)		3 ②								
Date:	6/3/15	6/4/15	6/5/15	6/8/15	6/9/15	6/10/15	6/12/15	6/15/15		
Time:	1100	1355	1500	1200	1050	1410	1425	1207		
Initials:	AB	AB	DAP	DAP	AB	AB	AB	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25°C~~ 22°C ③ prior to making the dilution series. ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~ ③

① AB 6/3/15 E, 1911 ② DAP for AB 6/5/15 E ③ DAP 10/19/15 E ④ DAP 12/11/15 E

CHRONIC CHEMICAL DATA ~~(INITIAL)~~ ALL ②

Project Number: 15001-468-010 QA: AR 12/9/15

Test Species: ~~*Rana*~~ Lithobates ② *pipiens*

%		Day 28 (Old)	Day 28 (New)	Day 30 (Old)	Day 33 (Old)	Day 35 (Old)	Day 35 (New)	Day 37 (Old)	Day 40 (Old)	Meter #	Remarks
Conc.:	MH	Rep D ↓	Comp ↓	Rep A ↓	Rep B ↓	Rep C ↓	Comp	Rep D ↓	Rep A ↓	All Conc.	
pH		8.0	8.4	8.1	8.1	8.0	8.4	7.9	8.4	Fm26	
D.O. (mg/L)		6.0	6.8	5.8	5.8	6.2	6.8	5.1	5.7	6	
Temp. (°C)		22.4	20.5	21.3	21.4	21.3	21.8	22.3	22.6	L-25	
Cond. (µS/cm)		344	294	363	464	410	315	377	491	16	
Hard. (mg/L)			90				90			TITR	
Alk. (mg/L)			59				58			TITR	
TRC (mg/L)			<0.02 6/17				<0.02 6/24			22	
NH_3 (mg/L)			<1.0 6/17				<1.0 6/24			HA#1	
Conc.:	1.28%										
pH		8.1	8.5	8.0	8.0	7.9	8.4	8.0	8.2		
D.O. (mg/L)		6.1	6.8	5.7	5.1	6.0	6.7	5.6	4.8		
Temp. (°C)		21.9	20.3	20.9	21.6	21.4	21.9	22.0	22.3		
Cond. (µS/cm)		485	413	467	556	555	447	499	571		
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
Conc.:	3.2%										
pH		8.0	8.4	7.9	8.0	7.8	8.4	7.8	8.2		
D.O. (mg/L)	5.9	~~21.8~~ ①	6.8	5.3	5.5	5.8	6.7	4.4	5.2		
Temp. (°C)		21.8	20.3	20.8	21.2	21.3	21.8	22.0	22.1		
Cond. (µS/cm)		640	549	632	711	728	631	670	742		
Conc.:	8%										
pH		8.1	8.4	7.9	8.1	7.9	8.3	7.9	8.3		
D.O. (mg/L)		6.2	6.8	5.5	5.9	6.0	6.8	5.3	5.4		
Temp. (°C)		22.0	20.3	21.2	20.9	21.3	21.8	22.4	22.6		
Cond. (µS/cm)		1092	1027	1045	1211	1207	1107	1136	1200		
Date:		6/17/15	6/17/15	6/19/15	6/22/15	6/24/15	6/24/15	6/26/15	6/29/15		
Time:		1015	1335	1410	1515	1050	1340	1045	1400		
Initials:		AB	AB	DAP	DAP	AB	AB	DAP	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25°C~~ 22°C ② prior to making the dilution series. ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~ ②

① AB 6/17/15 WP ② DAP 10/19/15 E ③ DAP 12/1/15 E

CHRONIC CHEMICAL DATA ~~(INITIAL)~~ ALL ②

Project Number: 15001-468-010 QA: MR 12/9/15

Test Species: ~~*Rana pipiens*~~ Lithobates ②

%	Day 28 (old)	Day 28 Comp (new)	Day 30 (old)	Day 33 (old)	Day 35 (old)	Day 35 (new)	Day 37 (old)	Day 40 (old)	Meter #	Remarks
Conc.: 20.0%	Rep D ↓	Day 28	Rep A ↓	Rep B ↓	Rep C ↓	Comp	Rep D ↓	Rep A ↓	All Conc.	
pH	8.2	8.2	8.0	8.2	7.8	8.2	7.7	8.3		
D.O. (mg/L)	6.3	6.8	5.9	6.1	6.0	6.8	3.8	5.6		
Temp. (°C)	21.8	20.4	21.3	21.0	21.3	21.9	21.8	22.6		
Cond. (µS/cm)	2001	2006	2133	2229	2254	2244	2105	2280		
Conc.: 50%										
pH	8.1	8.0	7.9	8.0	7.9	8.0	8.0	8.3		
D.O. (mg/L)	6.4	6.7	5.9	5.9	6.2	6.7	6.0	5.5		
Temp. (°C)	22.1	20.4	21.0	21.1	21.4	21.9	22.0	22.3		
Cond. (µS/cm)	4390	4210	4200	4730 ①	4740	4550	4480	4580		
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 75%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	6/17/15	6/17/15	6/19/15	6/22/15	6/24/15	6/24/15	6/26/15	6/29/15		
Time:	1015	1335	1410	1515	1050	1340	1045	1400		
Initials:	AB	AB	DAP	DAP	AB	AB	DAP	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25°C~~ 22°C ② prior to making the dilution series. ② ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~

① DAP 6/22/15 E [4730]

② DAP 10/19/15 E

③ DAP 12/11/15 E

ALL ②

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

Project Number: 15001-468-010 QA: ARV 2/9/15

Test Species: ~~*Rana pipiens*~~ Lithobates ②

%		Day 42 (old)	Day 42 (New)	Day 44 (old)	Day 47 (old)	Day 49 (old)	Day 49 (New)	Day 51 (old)	Day 54 (old)	Meter #	Remarks
Conc.:	MH	Rep B ↓	Comp	Rep E ↓	Rep D ↓	Rep A ↓	Comp	Rep B ↓	Rep C ↓	All Conc.	
pH		7.9	8.4	8.0	8.3 ①	8.3	8.3	8.0	8.1	Fm26	
D.O. (mg/L)		4.9	6.6	5.4	6.0	6.6	6.8	6.3	6.2	6	
Temp. (°C)		22.1	22.5	22.1	21.3	21.6	21.2	21.2	21.0	L25	
Cond. (µS/cm)		421	298	374	437	423	331	383	422	16	
Hard. (mg/L)							86			TITR	
Alk. (mg/L)							58			TITR	
TRC (mg/L)							0.03 7/8			22	
NH_3 (mg/L)							<1.0 7/8			HA#1	
Conc.:	1.28%										
pH		8.0	8.4	8.1	8.2	7.7	8.3	8.0	8.2		
D.O. (mg/L)		5.2	6.6	5.9	5.9	5.1	6.8	5.8	6.3		
Temp. (°C)		22.5	22.6	21.9	21.2	21.4	21.3	21.4	21.1		
Cond. (µS/cm)		566	424	489	548	580	410	486	470		
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
Conc.:	3.2%										
pH		8.0	8.4	8.1	8.1	8.0	8.2	7.9	8.2		
D.O. (mg/L)		5.2	6.6	6.0	5.2	5.7	6.8	5.7	6.1		
Temp. (°C)		22.4	22.7	21.8	21.2	21.4	21.4	21.2	20.9		
Cond. (µS/cm)		731	609	636	708	784	629	715	646		
Conc.:	8%										
pH		8.0	8.3	8.1	8.2	8.0	8.2	7.9	8.2		
D.O. (mg/L)		5.2	6.6	6.0	5.8	6.0	6.7	5.4	6.1		
Temp. (°C)		21.8	22.6	21.9	21.7	21.8	21.4	20.8	21.0		
Cond. (µS/cm)		1224	1029	1102	1192	1184	1092	1219	1082		
Date:		7/1/15	7/1/15	7/3/15	7/6/15	7/8/15	7/8/15	7/10/15	7/13/15		
Time:		1050	1335	0940	1110	1335	1350	1055	1135		
Initials:		AB	AB	DAP	DAP	AB	AB	DAP	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25°C~~ 22°C ② prior to making the dilution series. ② ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~

① On Day 47, chem. readings taken in Rep C in Control Treatment. No organisms remaining in Rep. D. DAP 7-6-15 ② DAP 10/19/15 E ③ DAP 12/10/15 E

ALL ②

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

Project Number: 15001-468-010 QA: AR 12/9/15

Test Species: ~~*Rana pipiens*~~ Lithobates ②

%	Day 42 (old)	Day 42 (New)	Day 44 (Old)	Day 47	Day 49 (old)	Day 49 (New)	Day 51 (old)	Day 54 (old)	Meter #	Remarks
Conc.: 20.0%	Rep B	comp	Rep C	Rep D	Rep A	comp	Rep B	Rep C	All Conc.	
pH	8.1	8.2	7.9	8.2	8.0	8.1	8.0	8.0		
D.O. (mg/L)	5.6	6.4	5.4	6.0	6.0	6.7	6.0	5.4		
Temp. (°C)	22.0	22.4	21.7	21.9	21.9	21.4	21.0	20.8		
Cond. (µS/cm)	2205	2167	2079	2210	2470	2210	2307	2109		
Conc.: 50%										
pH	7.8	8.0	7.8	8.2	7.9	7.9	7.9	8.1		
D.O. (mg/L)	4.9	6.4	5.3	6.1	5.8	6.7	5.6	6.2		
Temp. (°C)	22.0	22.5	22.0	21.3	21.9	21.4	21.3	20.9		
Cond. (µS/cm)	4440	4610	4390	4780	4950	4750	4920	4520		
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.:										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Conc.: 75%										
pH										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)										
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	7/1/15	7/1/15	7/3/15	7/6/15	7/8/15	7/8/15	7/8/15 ①10	7/13/15		
Time:	1050	1335	0940	1110	1335	1350	1055	1135		
Initials:	AB	AB	DAP	DAP	AB	AB	DAP	DAP		

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25°C~~ 22°C ② prior to making the dilution series. ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~ ②

① DAP 7/10/15 E ② DAP 10/19/15 E ③ DAP 12/11/15 E

ALL (1)

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

Project Number: 15001-468-010 QA: AR 12/9/15

Test Species: Lithobates (1) ~~Rana~~ pipiens

%		Day 56	Day 58	Day 61	Day 63	Day 65	Day 68	Day 70	Day	Meter #	Remarks
		old	old	old	old	old	old				
Conc.:	MH	Rep D ↓	Rep A ↓	Rep D ↓	Rep A ↓	Rep B ↓	Rep C ↓	Rep D ↓		All Conc.	
pH		8.1	8.2	8.2	8.2	8.2	8.3	8.1			
D.O. (mg/L)		6.3	6.0	5.1	4.8	5.6	5.3	4.6			
Temp. (°C)		21.2	20.5	21.6	22.0	21.4	23.5	21.1			
Cond. (µS/cm)		426	420	478	484	551	568	621			
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
Conc.:	1.28%										
pH		8.1	8.0	8.2	8.1	8.3	8.3	8.3			
D.O. (mg/L)		6.3	4.6	5.0	4.6	5.9	5.6	4.5			
Temp. (°C)		20.8	20.8	20.9	21.8	20.9	23.7	20.5			
Cond. (µS/cm)		563	492	594	596	650	647	762			
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
Conc.:	3.2%										
pH		8.0	8.0	8.1	8.1	8.2	8.2	8.2			
D.O. (mg/L)		5.5	5.0	4.8	5.0	5.8	3.6	3.4			
Temp. (°C)		20.9	21.0	20.8	21.2	20.8	23.3	20.9			
Cond. (µS/cm)		740	768	735	755	839	853	873			
Conc.:	8%										
pH		8.1	8.0	8.2	8.1	8.1	8.2	8.1			
D.O. (mg/L)		6.1	5.0	5.1	5.2	4.2	4.1	4.2			
Temp. (°C)		21.2	20.6	21.4	21.6	21.8	23.2	21.1			
Cond. (µS/cm)		1224	1255	1236	1262	1489	1623	1684			
Date:		7/15/15	7/17/15	7/20/15	7/22/15	7/24/15	7/27/15	7/29/15			
Time:		1235	1145	1125	1315	1000	1340	0825			
Initials:		DAP	DAP	DAP	DAP	DAP	DAP	DAP			

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to ~~25°C~~ 22°C (1) prior to making the dilution series. (1) ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~

(1) DAP 10/19/15 E (2) DAP 12/11/15 E

ALL (3)

CHRONIC CHEMICAL DATA ~~(INITIAL)~~

QA: AR 12/9/15

Project Number: 15001-468-010

Test Species: ~~*Rana pipiens*~~ Lithobates (3)

%		Day 56	Day 58	Day 61	Day 63	Day 65	Day 68	Day 70	Day	Meter #	Remarks
		old	old	old	old	old					
Conc.:	20.0%	Rep D	Rep A	Rep D	Rep A	Rep B	Rep C	Rep D		All Conc.	
pH		8.1	8.2	8.3	8.2	8.1	8.3	8.2			
D.O. (mg/L)		5.8	5.7	4.9	4.6	5.3	4.2	4.4			
Temp. (°C)		21.3	20.7	21.0	20.8	22.2	23.1	20.8			
Cond. (µS/cm)		2339	2225	2324	2364	2533	2521	2584			
Conc.:	50%										
pH		8.1	8.2	8.3	8.2	—					
D.O. (mg/L)		6.2	5.9	5.2	5.2	—					
Temp. (°C)		21.0	20.4	21.1	21.2	21.8					
Cond. (µS/cm)		4990	4650	4900	4980	—					
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Conc.:											
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Conc.:	75%										
pH											
D.O. (mg/L)											
Temp. (°C)											
Cond. (µS/cm)											
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
Date:		7/8/15	7/10/15	7/20/15	7/22/15	7/24/15	7/27/15 (2)	7/29/15			
Time:		1235	1145	1125	1315	1000	1340	0825			
Initials:		DAP	DAP	DAP	DAP	DAP	DAP	DAP			

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought ~~to 25°C~~ 22°C (3) prior to making the dilution series. ~~The temperature of resulting effluent dilution is assumed to also be 25°C.~~ (3)

(1) DAP 7/15/15 WP (2) DAP 7/27/15 E (3) DAP 10/18/15 E (4) DAP 12/1/15

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010
Test Species:	~~Rana~~ Lithobates pipiens ⑤

QA: AR 12/9/15

General Comments	FHM Bound used on Day 0 Random Chart: O Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 0	Test Solution Mixed at: 1000-1100 [Analytical samples collected - Each Treatm.] Test Organisms Added at: 1200 pH of (pH-adj.) NTO stock = 7.6 (FM26) DAP Temp: 24 °C Range: 24-25 °C	Fed @	DAP 5/20/15
Test Day 1	Real Time: 0284 °C Min-Max Range: 23-24 °C Live tadpoles @ GS 23-24 with ext. gills on left side still apparent	Fed @	DAP 5/21/15
Test Day 2	Real Time: 24 °C Min-Max Range: 24-26°C Started aeration @ 1630	Fed @ 1600 ~~1605~~ ② ~1/2 "Nip" ~0.025g	DAP 5/22/15
Test Day 3	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @	DAP 5/23/15
Test Day 4	Real Time: 24 °C Min-Max Range: 24-26 °C	Fed @	DAP 5/24/15
Test Day 5	Real Time: 24 °C Min-Max Range: 24-25 °C	Fed @ 1115 ~0.045g/chmbr	DAP 5/25/15
Test Day 6	Real Time: 25 °C Min-Max Range: 24-28 °C	Fed @	DAP 5/26/15
Test Day 7	Real Time: 26 °C Min-Max Range: 24-29 °C Organisms transferred to 2.5 gal. tanks from cups; tanks placed on 4 shelves - FHM Random chart IOTA	Fed @ 1425 0.077g ③ ~~2th bottle~~	DAP ③ 5/26/15
Test Day 8	Real Time: 26 °C Min-Max Range: 24-28 °C	Fed @	DAP 5/28/15

① DAP 5/21/15 E ② DAP 5/22/15 E ③ DAP 5/27/15 E ④ Chambers with ≥50% mortality are given next smallest amount (measuring spoon) DAP 5/27/15
⑤ DAP 10/19/15 E ⑥ DAP 12/11/15 E

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010	QA: AR 12/9/15
Test Species:	~~Rana~~ *pipiens* Lithobates ①	

General Comments	Random Chart: Iota	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 9	Real Time: 26 °C	Min-Max Range: 24-28 °C	Fed @ 1120 0.077g	DAP 5/29/15
Test Day 10	Real Time: 26 °C	Min-Max Range: 24-27 °C	Fed @	DAP 5/30/15
Test Day 11	Real Time: 26 °C	Min-Max Range: 24-26 °C	Fed @	DAP 5/31/15
Test Day 12	Real Time: 26 °C	Min-Max Range: 24-26 °C	Fed @ 1145 0.077g	DAP 6/1/15
Test Day 13	Real Time: 25 °C	Min-Max Range: 24-26 °C	Fed @	DAP 6/2/15
Test Day 14	Real Time: 26 °C	Min-Max Range: 25-28 °C	Fed @ 1520 0.1865g	DAP 6/3/15
Test Day 15	Real Time: 26 °C	Min-Max Range: 25-28 °C	Fed @	DAP 6/4/15
Test Day 16	Real Time: 24 °C	Min-Max Range: 24-26 °C	Fed @ 1535 0.1865g	DAP 6/5/15
Test Day 17	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/6/15

Note: Reduced feeding for ≥50% mortal. tanks, as needed. DAP

① DAP 10/19/15 E ② DAP 12/11/15 E

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010	QA: AR 12/9/15
Test Species:	~~Rana~~ pipiens	

Lithobates (1)

General Comments	Random Chart: Iota	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 18	Real Time: 24 °C	Min-Max Range: 24-26 °C	Fed @	DAP 6/7/15
Test Day 19	Real Time: 25 °C	Min-Max Range: 24-26 °C	Fed @ 1220 0.1865g	DAP 6/8/15
Test Day 20	Real Time: 24 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/9/15
Test Day 21	Real Time: 25 °C -Several tadpoles w hind limbs, Gosner 31-34 Limb buds not apparent on 50% treatm. tadpoles DAP	Min-Max Range: 24-25 °C	Fed @ 1615 0.1865 g	AB 6/10/15
Test Day 22	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/11/15
Test Day 23	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @ 1435 0.1865g	AB 6/12/15
Test Day 24	Real Time: 25 °C	Min-Max Range: 24-25 °C	Fed @	DAP 6/13/15
Test Day 25	Real Time: 24 °C	Min-Max Range: 24-27 °C	Fed @	DAP 6/14/15
Test Day 26	Real Time: 25 °C	Min-Max Range: 24-27 °C	Fed @ 1140 0.1865g	DAP 6/15/15

Note: Reduced Feeding (next smallest spoon) for tanks with < 50% of original # of organisms. DAP

(1) DAP 10/19/15 E (2) DAP 12/11/15 E

QA: AR 12/9/15

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010
Test Species:	~~*Rana*~~ *pipiens* Lithobates ②

General Comments	Random Chart: Iota	Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 27	Real Time: 22 °C	Min-Max Range: 21-25 °C	Fed @	DAP 6/16/15
Test Day 28	Real Time: 22 °C	Min-Max Range: 21-25 °C	Fed @ 1635 0.3055 g	AS 6/17/15
Test Day 29	Real Time: 21 °C	Min-Max Range: 21-24 °C	Fed @	DAP 6/18/15
Test Day 30	Real Time: 21 °C	Min-Max Range: 21-25 °C	Fed @ 1420 0.3055 g	DAP 6/19/15
Test Day 31	Real Time: 22 °C	Min-Max Range: 21-23 °C	Fed @	DAP 6/20/15
Test Day 32	Real Time: 22 °C	Min-Max Range: 21-24 °C	Fed @	DAP 6/21/15
Test Day 33	Real Time: 21 °C	Min-Max Range: 21-24 °C	Fed @ 1535 0.3055 g	DAP 6/22/15
Test Day 34	Real Time: 22 °C	Min-Max Range: 21-24 °C	Fed @	DAP 6/23/15
Test Day 35	Real Time: 22 °C	Min-Max Range: 21-25 °C	Fed @ 1600 0.611g ①	AS 6/24/15

① Twice the amount of food added per chamber (per Study Director's decision) because some tanks seemed devoid of previous food quickly (little debris remaining), which suggested more food was needed. 50% NTO chambers still received one "pinch" ~0.1865 g. DAP 6/24/15

② DAP 10/08/15 E ③ DAP 03/11/15 E

QA: AR 12/9/15

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010
Test Species:	~~*Rana pipiens*~~ Lithobates ②

General Comments	Random Chart: Iota Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 36	Real Time: 23 °C Min-Max Range: 21-25 °C Some animals in several tanks were at surface, apparently gulping air, therefore, increased aeration in several tanks. DAP @ 1555	~~Fed @~~ ① DO in cont, RpB = 4.8 mg/L	DAP 6/25/15
Test Day 37	Real Time: 23 °C Min-Max Range: 21-25 °C	Fed @ 1120 0.3055 g	DAP 6/26/15
Test Day 38	Real Time: 22 °C Min-Max Range: 22-25 °C	Fed @	DAP 6/27/15
Test Day 39	Real Time: 22 °C Min-Max Range: 22-25 °C	Fed @	DAP 6/28/15
Test Day 40	Real Time: 23 °C Min-Max Range: 23-26 °C -3 tadpoles with front legs (See biological data). DAP	Fed @ 1445 0.3055 g	DAP 6/29/15
Test Day 41	Real Time: 23 °C Min-Max Range: 22-25 °C -3 add. tadpoles with front legs (See biol. data) DAP	Fed @	DAP 6/30/15
Test Day 42	Real Time: 23 °C Min-Max Range: 22-25 °C Emergence chambers Fed 0.045g DAP	Fed @ 1600 0.3055 g	AB 7/1/15
Test Day 43	Real Time: 23 °C Min-Max Range: 21-25 °C Light measurement @ approx water level in chambers @ 1455 DAP - See Below	Fed @	DAP 7/2/15
Test Day 44	Real Time: °C Min-Max Range: °C Emergence chambers Fed 0.045g - DAP	Fed @ 1025 0.3055g	DAP 7/3/15

① DAP 6/25/15 CF ② DAP 10/15/15 E ③ DAP 12/10/15 E

Note: Reduced Feeding (next smallest spoon) for tanks with <50% of original # of organisms, DAP

Light Measurements (Foot Candles) Left shelf

1					1	Right shelf
2					2	
3	195	219	195	191	3	
4	190	175	205	196	4	

$\bar{X}$ = 195.75 FC

DAILY TOXICITY TEST LOG

Project Number: 15001-468-010 QA: AR 12/9/15
Test Species: ~~Rana~~ *pipiens*
Lithobates ①

General Comments	Random Chart: Iota Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 45	Real Time: 22 °C Min-Max Range: 21-25 °C	Fed @	DAP 7/4/15
Test Day 46	Real Time: 21 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/5/15
Test Day 47	Real Time: 22 °C Min-Max Range: 21-25 °C Emergence chambers fed 0.045g. DAP	Fed @ 1215 0.3055g	DAP 7/6/15
Test Day 48	Real Time: 23 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/7/15
Test Day 49	Real Time: 23 °C Min-Max Range: 22-24 °C Emergence chambers fed 0.045g - AB	Fed @ 1620 0.3055g	AB 7/8/15
Test Day 50	Real Time: 22 °C Min-Max Range: 21-26 °C	Fed @	DAP 7/9/15
Test Day 51	Real Time: 22 °C Min-Max Range: 21-24 °C Emerg. chambers fed 0.045g DAP	Fed @ 1100 0.3055g	DAP 7/10/15
Test Day 52	Real Time: 21 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/11/15
Test Day 53	Real Time: 22 °C Min-Max Range: 21-26 °C	Fed @	7/12/15 DAP

Note: Reduced feeding (next smallest spoon) for tanks with <50% of original # of organisms. DAP

① DAP 10/19/15 E ② DAP 12/11/15 E

DAILY TOXICITY TEST LOG

Project Number:	15001-468-010	QA: AR 12/9/15
Test Species:	~~Rana pipiens~~ Lithobates ⑤	

General Comments	Random Chart: Tota Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 54	Real Time: 22 °C Min-Max Range: 21-26 °C Emerg. chambers fed 0.045g DAP	Fed @ 1130 0.3055g	DAP 7/13/15
Test Day 55	Real Time: 22 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/14/15
Test Day 56	Real Time: 22 °C Min-Max Range: 21-24 °C - Water was not renewed. DAP 7/15/15 Emerg. Chambers fed 0.045g DAP	Fed @ 1300 0.3055g	DAP 7/15/15
Test Day 57	Real Time: 22 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/16/15
Test Day 58	Real Time: 22 °C Min-Max Range: 21-24 °C Emerg. chambers fed 0.045g. DAP	Fed @ 1320 0.1865g + 0.3055g ①	DAP 7/17/15
Test Day 59	Real Time: 22 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/18/15
Test Day 60	Real Time: 22 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/19/15
Test Day 61	Real Time: 22 °C Min-Max Range: 19-24 °C Emerg. chambers fed 0.045g DAP ③ 4	Fed @ 1128 0.1865g + 0.3055g ④	DAP 7/20/15
Test Day ~~63~~ ③ 2	Real Time: 21 °C Min-Max Range: 21-25 °C	Fed @	DAP 7/21/15

Note: Reduced feeding (next smallest spoon) for tanks with ≤50% of original # of organisms. DAP ⑤ DAP 10/19/15 E ⑥ DAP 12/11/15 E

① Only 20% Reps B + ② D receiving full amount of food (0.3055g), all others with ≤50% of original # of organisms, therefore are receiving ≈ 1/2 of full amount (the next smallest spoon) which is 0.1865g. DAP 7/17/15

② DAP 7/17/15 E ③ DAP 7/20/15 E

④ Only 20% D Fed full amount (0.3055g), all other chambers rec. 0.1865g (next smallest spoon). DAP 7/20/15

QA Form No. 055
Revision 3
Effective 02/14

DAILY TOXICITY TEST LOG

Project Number: 15001-468-010 QA: AR 12/9/15
Test Species: ~~*Rana pipiens*~~ Lithobates ②

General Comments	Random Chart: Tota Min/Max Thermometer # M-15	Feeding Grnd Tetramin	Initials/Date
Test Day 63	Real Time: 22 °C Min-Max Range: 21-24 °C Emerg. chmbrs received 0.045g. DAP	Fed @ 1340 0.1865g ①	DAP 7/23/15
Test Day 64	Real Time: 22 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/23/15
Test Day 65	Real Time: 22 °C Min-Max Range: 21-24 °C Emerg. chmbrs. received 0.045g DAP	Fed @ 1145 0.1865g ①	DAP 7/24/15
Test Day 66	Real Time: 22 °C Min-Max Range: 21-25 °C	Fed @	DAP 7/25/15
Test Day 67	Real Time: 22 °C Min-Max Range: 22-26 °C	Fed @	DAP 7/26/15
Test Day 68	Real Time: 22 °C Min-Max Range: 22-26 °C Emerg. chmbrs received 0.045g DAP	Fed @ 1530 0.1865g	DAP 7/27/15
Test Day 69	Real Time: 22 °C Min-Max Range: 24-26 °C Light measurements taken @ 1620 DAP See Below	Fed @	DAP 7/28/15
Test Day 70	Real Time: 21 °C Min-Max Range: 21-24 °C Tadpole Exposures terminated. Emergence chmbrs Fed 0.045g. DAP	Fed @	DAP 7/29/15
Test Day 71	Real Time: 22 °C Min-Max Range: 21-24 °C	Fed @	DAP 7/30/15

① All remaining tanks have <50% original organisms. DAP 7/22/15
② DAP 10/12/15 E ③ DAP 12/11/15 E

Light Measurements (Foot Candles)

Left shelf	1				1
	2				2
	3	189	197	198	196	3
	4	210	174	216	204	4

Right shelf

$\bar{x}$ = 198 FC

Activities Log

Blot wts of Emerged frogs

Date	Time	Initials	Notes			
7/3/15	1500	DAP	1.28C	1.7643 g	✓	✓
			8 A	1.2452 g	✓	✓
			O D	1.4832 g	✓	✓
			O A	1.8610 g	✓	✓
			3.2 D	1.5470 g	✓	✓
7/5/15	1240	DAP	C C	1.8389 g	✓	✓
			C D	1.8969 g	✓	✓
			3.2 C	1.8653 g	✓	✓
			8 C	1.7632 g	✓	✓
7/6/15	1630	DAP	C B	1.4603 g	✓	✓
7/8/15	1550	DAP	C D	1.8387 g	✓	✓
			C A	1.7893 g	✓	✓
			C B	1.4727 g	✓	✓
			C B	1.6861 g	✓	✓
			8 D	2.4795 g	✓	✓
			3.2 A	1.9421 g	✓	✓

Activities Log

Blot wts of Emerged Frogs

Date	Time	Initials	Notes			
7/9/15	1430	DAP	1.28 B	1.5968 g	✓	✓
7/10/15	1445	DAP	1.28 O	2.1624 g	✓	✓
			8 A	1.3369 g	✓	✓
			c D	2.0515 g	✓	✓
			3.2 D	1.5380 g	✓	✓
			3.2 D	1.6277 g	✓	✓
			20 C	1.6099 g	✓	✓
7/11/15	0930	DAP	1.28 A	2.1852 g	✓	✓
			1.28 A	1.7642 g	✓	✓
			3.2 B	2.0966 g	✓	✓
7/12/15	1455	DAP	c B	1.7373 g	✓	✓
			8 A	1.3096 g	✓	✓
			8 B	2.1169 g	✓	✓
			8 B	1.8009 g	✓	✓
			8 D	2.6554 g	✓	✓

Activities Log

Blot wts of Emerged Frogs

Date	Time	Initials	Notes			
7/13/15	1330	DAP	C A	1.1047 g	✓	✓
			C C	1.6339 g	✓	✓
			3.2 B	2.0005 g	✓	✓
			8 B	1.2181 g	✓	✓
7/14/15	1520	DAP	1.28 D	2.1422 g	✓	✓
			3.2 C	2.2845 g	✓	✓
7/15/15	1400	DAP	C B	1.6782 g	✓	✓
			3.2 A	1.9462 g	✓	✓
			8 A	2.0336 g	✓	✓
			8 C	1.7250 g	✓	✓
7/16/15	0930	DAP	C C	2.2797 g	✓	✓
			1.28 B	1.3622 g	✓	✓
			3.2 B	2.2199 g	✓	✓

Activities Log

Date	Time	Initials	Notes
7/17/15	1220	DAP	1.28 B 1.1246 g ✓ ✓
			1.28 D 2.1488 g ✓ ✓
			3.2 A 2.7468 g ✓ ✓
			20 A 1.9779 g ✓ ✓
			20 C 1.8772 g ✓ ✓
7/19/15	0925	DAP	3.2 D 1.5265 g ✓ ✓
			20 D 2.2320 g ✓ ✓
7/20/15	1215	DAP	3.2 C 2.0040 g ✓ ✓
			20 A 1.9433 g ✓ ✓
7/21/15	1125	DAP	8 C 1.6336 g ✓ ✓
			20 C 2.3670 g ✓ ✓
7/23/15	1310	DAP	1.28 A 1.1811 g ✓ ✓
			1.28 A 1.6602 g ✓ ✓
			20 B 2.4955 g ✓ ✓
7/24/15	1130	DAP	8 B 1.6917 g ✓ ✓
			20 D 2.0912 g ✓ ✓

Activities Log

Date	Time	Initials	Notes
7/27/15	1410	DAP	1.28 C 1.4435 g ✓ ✓
			8 C 1.4130 g ✓ ✓
			8 C 1.7502 g ✓ ✓
7/28/15	1515	DAP	C C 1.5285 g ✓ ✓
7/30/15	1730	DAP	20B 1.9305 g ✓
7/31/15	1450	DAP	20B 1.7158 g ✓

CETIS Analytical Report

Lithobates pipiens 70-d

Report Date:	15 Dec-15 09:25 (p 1 of 2)
Test Code:	468-010-28sur | 08-7136-4333

~~Fathead Minnow 96-h~~ Acute Survival Test 14-Day

TRE Environmental Strategies, LLC

Analysis ID:	05-6075-8537	**Endpoint:**	~~72h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:25	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	03-3027-9319	**Test Type:**	Survival ~~(96h)~~ 14-D	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	<24

Sample ID:	09-4549-8235	**Code:**	385B287B	**Client:**	Confidential
Sample Date:	~~20 May-15~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~20 May-15 09:10~~	**Source:**	Research		
Sample Age:	~~12h~~	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	12.8%	3070	7775	4886	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		480	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		1230	16	10	1	6	0.6105	Asymp	Non-Significant Effect
		3070	12	10	1	6	0.1424	Asymp	Non-Significant Effect
		7775*	10	10	0	6	0.0417	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.576244	0.3152488	5	46.41	<0.0001	Significant Effect
Error	0.1222699	0.00679277	18			
Total	1.698514		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.556	4.25	0.7321	Equal Variances
Variances	Levene Equality of Variance	5.004	4.25	0.0048	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8739	0.884	0.0063	Non-normal Distribution

~~72h~~ 14-D Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
201		4	1	1	1	1	1	1	0	0.0%	0.0%
480		4	1	1	1	1	1	1	0	0.0%	0.0%
1230		4	0.95	0.7909	1	1	0.8	1	0.05	10.5%	5.0%
3070		4	0.85	0.6909	1	0.8	0.8	1	0.05	11.8%	15.0%
7775		4	0.35	0.1909	0.5091	0.4	0.2	0.4	0.05	28.6%	65.0%
11350		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
201		4	1.338	1.317	1.36	1.345	1.318	1.345	0.006791	1.01%	0.51%
480		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
1230		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	4.43%
3070		4	1.167	0.9772	1.356	1.107	1.107	1.345	0.05953	10.2%	13.3%
7775		4	0.6295	0.4536	0.8053	0.6847	0.4636	0.6847	0.05527	17.6%	53.2%
11350		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.2%

AMR 12/15/15 E

CETIS Analytical Report

Lithobates Pipiens 70-d

~~Fathead Minnow 96-h~~ Acute Survival Test 14-Day

TRE Environmental Strategies, LLC

Analysis ID:	05-6075-8537	**Endpoint:**	~~72h~~ 14-D Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:25	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

~~72h~~ 14-Day Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
201		1	1	1	1
480		1	1	1	1
1230		1	1	1	0.8
3070		0.8	0.8	1	0.8
7775		0.4	0.4	0.2	0.4
11350		0	0	0	0

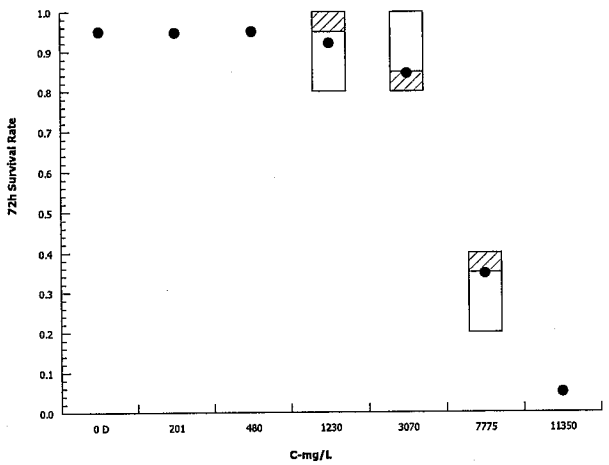
Angular (Corrected) Transformed Detail

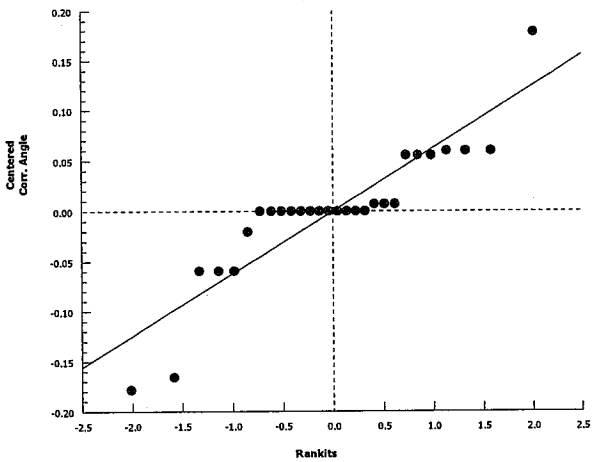
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.345	1.345	1.345	1.345
201		1.318	1.345	1.345	1.345
480		1.345	1.345	1.345	1.345
1230		1.345	1.345	1.345	1.107
3070		1.107	1.107	1.345	1.107
7775		0.6847	0.6847	0.4636	0.6847
11350		0.2255	0.2255	0.2255	0.2255

~~72h~~ 14-Day Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
201		4/4	5/5	5/5	5/5
480		5/5	5/5	5/5	5/5
1230		5/5	5/5	5/5	4/5
3070		4/5	4/5	5/5	4/5
7775		2/5	2/5	1/5	2/5
11350		0/5	0/5	0/5	0/5

Graphics





CETIS Analytical Report

Lithobates pipiens 70-Day

~~Fathead Minnow 96-h~~ Acute Survival Test 14-Day

Report Date:	15 Dec-15 09:25 (p 1 of 3)
Test Code:	468-010-28sur | 08-7136-4333

TRE Environmental Strategies, LLC

Analysis ID:	14-1236-1018	**Endpoint:**	~~72h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:25	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Batch ID:	03-3027-9319	**Test Type:**	Survival (~~96h~~) 14-Day	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	<24

Sample ID:	09-4549-8235	**Code:**	385B287B	**Client:**	Confidential
Sample Date:	~~20 May-15~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~20 May-15 09:10~~	**Source:**	Research		
Sample Age:	~~12h~~	**Station:**			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
60	-28.92	64.84	67.84	3.702	0.2739	0.7863	15.57	2.84	0.0000	Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	1786	910.1	2547
LC10	2246	1279	3058
LC15	2622	1604	3470
LC20	2964	1915	3848
LC25	3294	2224	4216
LC40	4296	3186	5397
LC50	5040	3890	6366

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	2.99E-07	9.61E-05	-0.00019	0.000189	0.003109	0.9975	Non-Significant Parameter
Slope	3.651	0.656	2.365	4.937	5.566	<0.0001	Significant Parameter
Intercept	-13.52	2.461	-18.34	-8.693	-5.492	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	80.28925	80.28925	1	101.4	<0.0001	Significant
Lack of Fit	14.80884	3.702209	4	15.57	<0.0001	Significant
Pure Error	4.993706	0.237796	21			
Residual	19.80254	0.792102	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	19.8	37.65	0.7571	Non-Significant Heterogenity
	Likelihood Ratio GOF	12.63	37.65	0.9807	Non-Significant Heterogenity
Variances	Mod Levene Equality of Variance	0.8242	2.573	0.5640	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.6661	0.9264	<0.0001	Non-normal Distribution
	Anderson-Darling A2 Normality	3.319	2.492	<0.0001	Non-normal Distribution

CETIS Analytical Report

Lithobates pipiens 70-Day

~~Fathead Minnow 96-h~~ Acute Survival Test 14-Day

TRE Environmental Strategies, LLC

Analysis ID:	14-1236-1018	Endpoint:	~~72h~~ 14-Day Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	15 Dec-15 9:25	Analysis:	Linear Regression (MLE)	Official Results:	Yes

~~72h~~ 14-Day Survival Rate Summary

C-mg/L	Control Type	Count	Calculated Variate(A/B) Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	1	1	1	0	0	0.0%	0.0%	20	20
201		4	1	1	1	0	0	0.0%	0.0%	19	19
480		4	1	1	1	0	0	0.0%	0.0%	20	20
1230		4	0.95	0.8	1	0.05	0.1	10.5%	5.0%	19	20
3070		4	0.85	0.8	1	0.05	0.1	11.8%	15.0%	17	20
7775		4	0.35	0.2	0.4	0.05	0.1	28.6%	65.0%	7	20
11350		4	0	0	0	0	0		100.0%	0	20

~~72h~~ 14-Day Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	1
201		1	1	1	1
480		1	1	1	1
1230		1	1	1	0.8
3070		0.8	0.8	1	0.8
7775		0.4	0.4	0.2	0.4
11350		0	0	0	0

~~72h~~ 14-Day Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	5/5
201		4/4	5/5	5/5	5/5
480		5/5	5/5	5/5	5/5
1230		5/5	5/5	5/5	4/5
3070		4/5	4/5	5/5	4/5
7775		2/5	2/5	1/5	2/5
11350		0/5	0/5	0/5	0/5

CETIS Analytical Report

Lithobates pipiens 70-Day

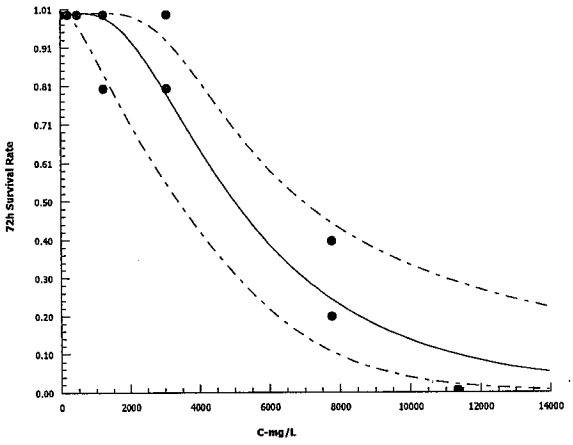
Test Code: 468-010-28sur | 08-7136-4333

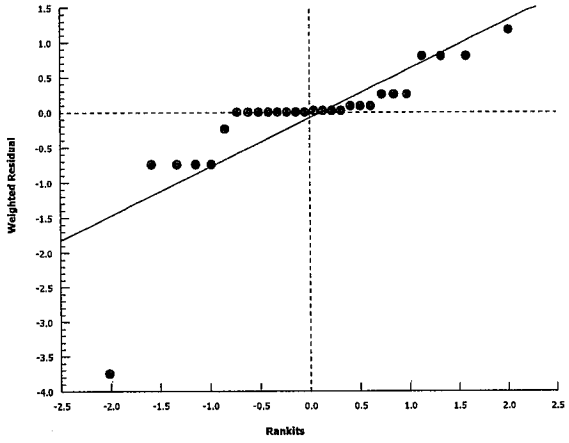
~~Fathead Minnow 96-h~~ Acute Survival Test 14-Day

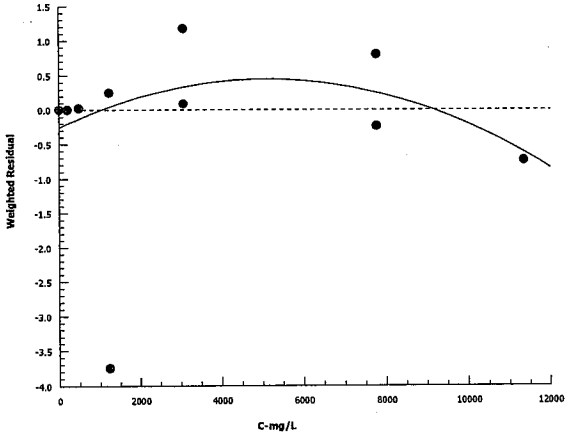
TRE Environmental Strategies, LLC

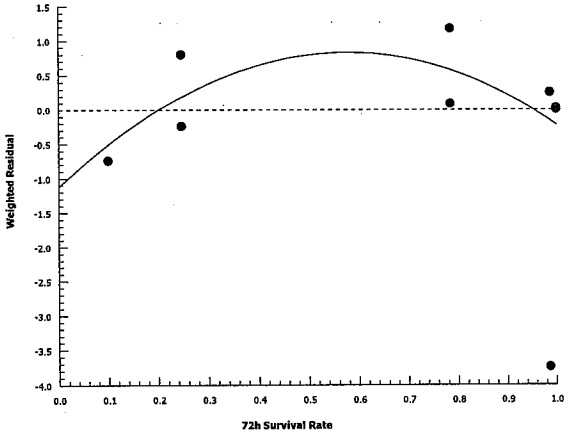
Analysis ID:	14-1236-1018	**Endpoint:**	~~72h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:25	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Graphics Log-Normal [NED=A+B*log(X)]









CETIS Analytical Report

Lithobates pipiens 70-D

~~Fathead Minnow 96-h~~ Acute Survival Test 28-Day

Report Date:	15 Dec-15 09:27 (p 1 of 2)		
Test Code:	468-010-28sur | 08-7136-4333		
TRE Environmental Strategies, LLC			

Analysis ID:	10-7612-8316	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:27	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	03-3027-9319	**Test Type:**	Survival ~~(96h)~~ 28-D	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	<24

Sample ID:	09-4549-8235	**Code:**	385B287B	**Client:**	Confidential
Sample Date:	~~20 May-15~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~20 May-15 09:10~~	**Source:**	Research		
Sample Age:	~~12h~~	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	14.5%	3070	7775	4886	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		201	-0.7795	2.41	0.163	6	0.9692	CDF	Non-Significant Effect
		480	-0.8799	2.41	0.163	6	0.9762	CDF	Non-Significant Effect
		1230	0	2.41	0.163	6	0.8333	CDF	Non-Significant Effect
		3070	1.76	2.41	0.163	6	0.1547	CDF	Non-Significant Effect
		7775*	11.33	2.41	0.163	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.035151	0.4070301	5	44.46	<0.0001	Significant Effect
Error	0.1648008	0.009155599	18			
Total	2.199952		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.3565	4.25	0.8713	Equal Variances
Variances	Levene Equality of Variance	3.209	4.25	0.0303	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9267	0.884	0.0822	Normal Distribution

~~96h~~ 28-Day Survival Rate Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.95	0.7909	1	1	0.8	1	0.05	10.5%	0.0%
201		4	1	1	1	1	1	1	0	0.0%	-5.26%
480		4	1	1	1	1	1	1	0	0.0%	-5.26%
1230		4	0.95	0.7909	1	1	0.8	1	0.05	10.5%	0.0%
3070		4	0.85	0.6909	1	0.8	0.8	1	0.05	11.8%	10.5%
7775		4	0.25	0.09088	0.4091	0.2	0.2	0.4	0.05	40.0%	73.7%
11350		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
201		4	1.338	1.317	1.36	1.345	1.318	1.345	0.006791	1.01%	-4.1%
480		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-4.63%
1230		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
3070		4	1.167	0.9772	1.356	1.107	1.107	1.345	0.05953	10.2%	9.26%
7775		4	0.5189	0.343	0.6948	0.4636	0.4636	0.6847	0.05527	21.3%	59.6%
11350		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	82.5%

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CETIS Analytical Report

Lithobates pipiens 70-Day

~~Fathead Minnow 96-h~~ Acute Survival Test 28-Day

TRE Environmental Strategies, LLC

Analysis ID:	10-7612-8316	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:27	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

28-Day ~~96h~~ Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.8
201		1	1	1	1
480		1	1	1	1
1230		1	1	1	0.8
3070		0.8	0.8	1	0.8
7775		0.2	0.2	0.2	0.4
11350		0	0	0	0

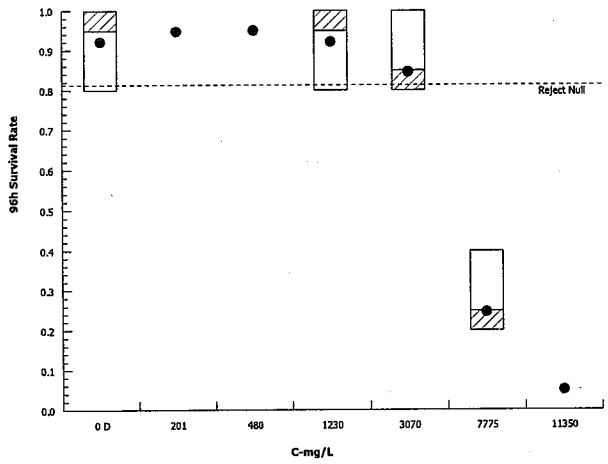
Angular (Corrected) Transformed Detail

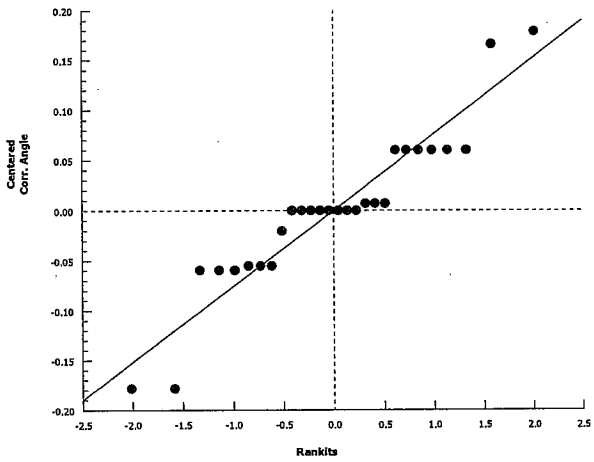
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.345	1.345	1.345	1.107
201		1.318	1.345	1.345	1.345
480		1.345	1.345	1.345	1.345
1230		1.345	1.345	1.345	1.107
3070		1.107	1.107	1.345	1.107
7775		0.4636	0.4636	0.4636	0.6847
11350		0.2255	0.2255	0.2255	0.2255

28-Day ~~96h~~ Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	4/5
201		4/4	5/5	5/5	5/5
480		5/5	5/5	5/5	5/5
1230		5/5	5/5	5/5	4/5
3070		4/5	4/5	5/5	4/5
7775		1/5	1/5	1/5	2/5
11350		0/5	0/5	0/5	0/5

Graphics





CETIS Analytical Report

Lithobates pipiens 70-Day

~~Fathead Minnow 96-h~~ Acute Survival Test 28-Day

Report Date:	15 Dec-15 09:27 (p 1 of 3)
Test Code:	468-010-28sur | 08-7136-4333

TRE Environmental Strategies, LLC

Analysis ID:	16-8449-8672	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:27	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Batch ID:	03-3027-9319	**Test Type:**	Survival ~~(96h)~~ 28-Day	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	<24

Sample ID:	09-4549-8235	**Code:**	385B287B	**Client:**	Confidential
Sample Date:	~~20 May-15~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~20 May-15 09:10~~	**Source:**	Research		
Sample Age:	~~12h~~	**Station:**			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.05	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
9	-30.12	67.24	70.24	3.723	0.189	0.8137	5.883	2.84	0.0024	Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	2580	1332	3525
LC10	3022	1719	3983
LC15	3362	2037	4334
LC20	3660	2328	4643
LC25	3936	2606	4932
LC40	4728	3432	5797
LC50	5279	4014	6449

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.02587	0.01794	-0.00929	0.06103	1.442	0.1617	Non-Significant Parameter
Slope	5.291	1.084	3.166	7.417	4.88	<0.0001	Significant Parameter
Intercept	-19.7	4.128	-27.79	-11.61	-4.772	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	84.33519	84.33519	1	119.9	<0.0001	Significant
Lack of Fit	9.291843	2.322961	4	5.883	0.0024	Significant
Pure Error	8.29226	0.39487	21			
Residual	17.5841	0.703364	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	17.58	37.65	0.8595	Non-Significant Heterogenity
	Likelihood Ratio GOF	13.48	37.65	0.9700	Non-Significant Heterogenity
Variances	Mod Levene Equality of Variance	0.5812	2.573	0.7413	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7365	0.9264	<0.0001	Non-normal Distribution
	Anderson-Darling A2 Normality	3.169	2.492	<0.0001	Non-normal Distribution

CETIS Analytical Report

Lithobates Pipiens 70-day

~~Fathead Minnow 96-h~~ Acute Survival Test 28-Day

TRE Environmental Strategies, LLC

Analysis ID:	16-8449-8672	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:27	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

~~96h~~ 28-Day Survival Rate Summary

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
			Calculated Variate(A/B)								
0	Dilution Water	4	0.95	0.8	1	0.05	0.1	10.5%	0.0%	19	20
201		4	1	1	1	0	0	0.0%	-5.26%	19	19
480		4	1	1	1	0	0	0.0%	-5.26%	20	20
1230		4	0.95	0.8	1	0.05	0.1	10.5%	0.0%	19	20
3070		4	0.85	0.8	1	0.05	0.1	11.8%	10.5%	17	20
7775		4	0.25	0.2	0.4	0.05	0.1	40.0%	73.7%	5	20
11350		4	0	0	0	0	0		100.0%	0	20

~~96h~~ 28-Day Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.8
201		1	1	1	1
480		1	1	1	1
1230		1	1	1	0.8
3070		0.8	0.8	1	0.8
7775		0.2	0.2	0.2	0.4
11350		0	0	0	0

~~96h~~ 28-Day Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	5/5	5/5	5/5	4/5
201		4/4	5/5	5/5	5/5
480		5/5	5/5	5/5	5/5
1230		5/5	5/5	5/5	4/5
3070		4/5	4/5	5/5	4/5
7775		1/5	1/5	1/5	2/5
11350		0/5	0/5	0/5	0/5

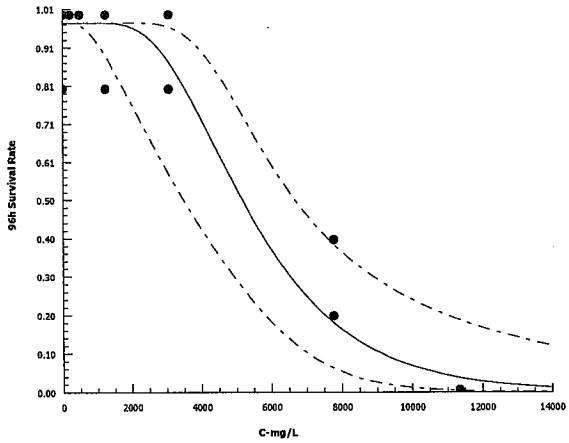
Lithobates pipiens 70-Day

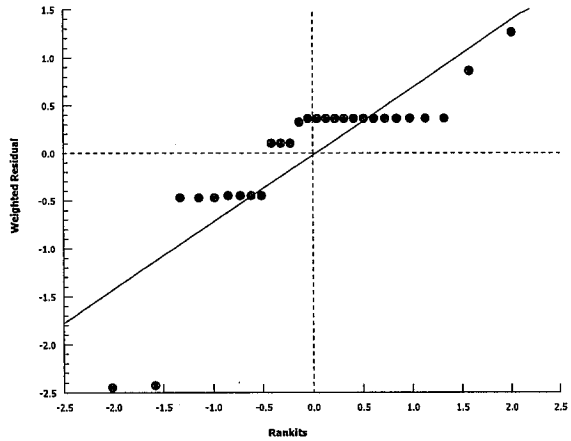
~~Fathead Minnow 96-h~~ Acute Survival Test 28-Day

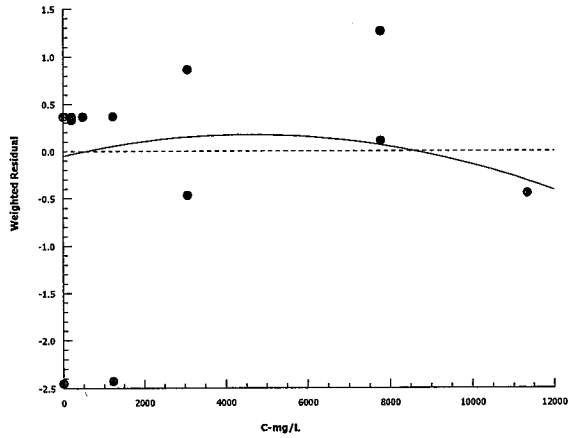
TRE Environmental Strategies, LLC

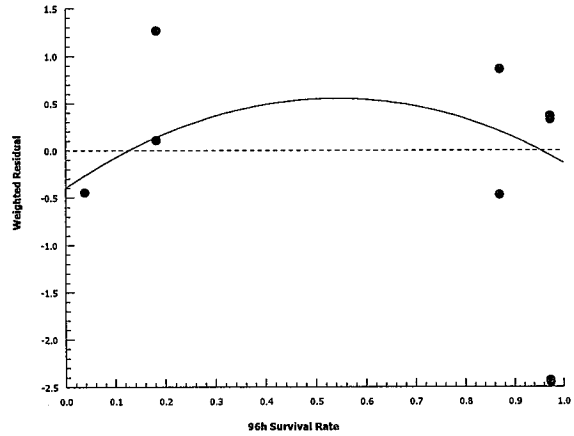
Analysis ID:	16-8449-8672	**Endpoint:**	~~96h~~ Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Dec-15 9:27	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Graphics Log-Normal [NED=A+B*log(X)]









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CETIS Analytical Report

Lithobates pipiens 70-day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test 7-day

Report Date:	24 Feb-16 14:15 (p 1 of 2)
Test Code:	468-010-70limb | 03-1680-6292

TRE Environmental Strategies, LLC

Analysis ID:	13-6589-1834	Endpoint:	~~Sd~~ Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:14	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~7d~~ 70d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	<224

Sample ID:	09-4549-8235	Code:	385B287B	Client:	Confidential
Sample Date:	~~20 May-15~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~20 May-15 09:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	35.6%	3338	8382	5290	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	0.7654	2.36	0.361	6	0.4855	CDF	Non-Significant Effect
0.04		517	-0.02783	2.36	0.361	6	0.8089	CDF	Non-Significant Effect
0.04		1346	0.6889	2.36	0.361	6	0.5199	CDF	Non-Significant Effect
0.04		3338	2.219	2.36	0.361	6	0.0639	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3127117	0.07817793	4	1.663	0.2105	Non-Significant Effect
Error	0.7049465	0.04699643	15			
Total	1.017658		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.776	4.89	0.1863	Equal Variances
Variances	Levene Equality of Variance	4.043	4.89	0.0203	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9164	0.866	0.0846	Normal Distribution

~~Sd~~ Survival Rate Summary

70-Day

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	0.9	0.7163	1	0.9	0.8	1	0.05774	12.8%	0.0%
190		4	0.8	0.4325	1	0.8	0.6	1	0.1155	28.9%	11.1%
517		4	0.9	0.5818	1	1	0.6	1	0.1	22.2%	0.0%
1346		4	0.8	0.3499	1	0.9	0.4	1	0.1414	35.4%	11.1%
3338		4	0.6	0.5999	0.6002	0.6	0.6	0.6	0	0.0%	33.3%
8382		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	1.226	1.007	1.445	1.226	1.107	1.345	0.06874	11.2%	0.0%
190		4	1.109	0.6991	1.519	1.102	0.8861	1.345	0.1288	23.2%	9.57%
517		4	1.23	0.8661	1.596	1.345	0.8861	1.345	0.1148	18.7%	-0.35%
1346		4	1.121	0.6249	1.616	1.226	0.6847	1.345	0.1558	27.8%	8.61%
3338		4	0.8861	0.8859	0.8862	0.8861	0.8861	0.8861	0	0.0%	27.7%
8382		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	81.6%

CETIS Analytical Report

Lithobates pipiens 7C-Day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test 7C-Day

Report Date:	24 Feb-16 14:15 (p 2 of 2)
Test Code:	468-010-70limb . 03-1680-5292
	TRE Environmental Strategies, LLC

Analysis ID:	13-6589-1834	Endpoint:	~~7-d~~ 7C Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:14	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

~~7-d~~ 7C-Day Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.8	1	1	0.8
190		1	0.6	0.6	1
517		1	1	0.6	1
1346		0.8	1	1	0.4
3338		0.6	0.6	0.6	0.6
8382		0	0	0	0

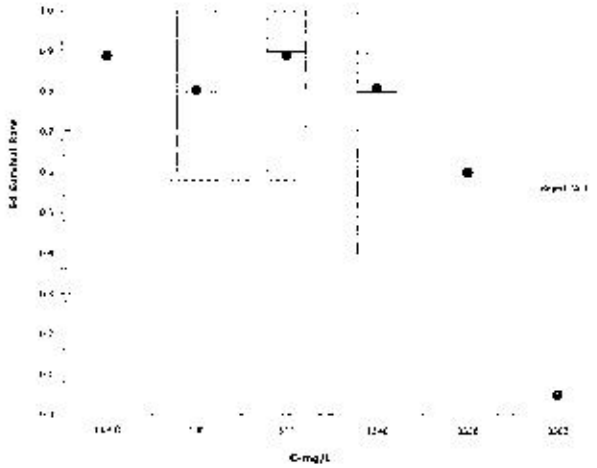
Angular (Corrected) Transformed Detail

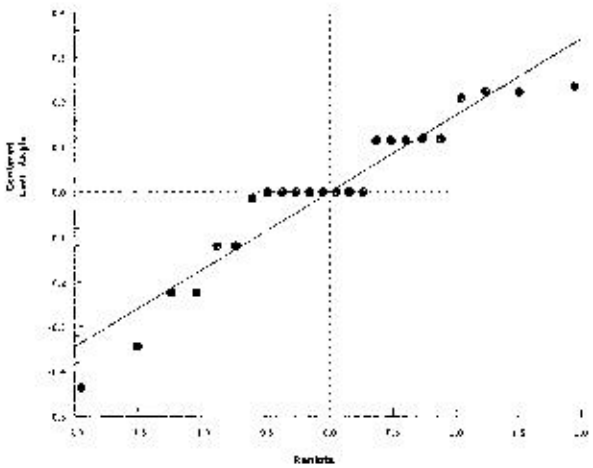
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	1.107	1.345	1.345	1.107
190		1.318	0.8861	0.8861	1.345
517		1.345	1.345	0.8861	1.345
1346		1.107	1.345	1.345	0.6847
3338		0.8861	0.8861	0.8861	0.8861
8382		0.2255	0.2255	0.2255	0.2255

~~7-d~~ 7C-Day Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	4/5	5/5	5/5	4/5
190		4/4	3/5	3/5	5/5
517		5/5	5/5	3/5	5/5
1346		4/5	5/5	5/5	2/5
3338		3/5	3/5	3/5	3/5
8382		0/5	0/5	0/5	0/5

Graphics





DM 2/24/16

CETIS Analytical Report

Lithobates pipiens 10-Day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	24 Feb-16 14:15 (p 1 of 2)		
Test Code:	468-010-70limb | 03-1580-6292		
TRE Environmental Strategies, LLC			

Analysis ID:	16-0451-4839	Endpoint:	~~7d~~ 10-Day Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:15	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival (~~24~~ 10 d)	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~324~~

Sample ID:	09-4549-8235	Code:	385B287B	Client:	Confidential
Sample Date:	~~20 May-15~~	Material:	~~Not Applicable~~ NTC	Project:	Special Studies
Receive Date:	~~20 May-15 00:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0.1	8.14%	3.566	0.05693	3670	2798	4814

~~7d~~ 10 d Survival Rate Summary

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
						Calculated Variate(A/B)					
0.04	Dilution Water	4	0.9	0.8	1	0.05774	0.1155	12.8%	0.0%	18	20
190		4	0.8	0.6	1	0.1155	0.2309	28.9%	11.1%	15	19
517		4	0.9	0.6	1	0.1	0.2	22.2%	0.0%	18	20
1346		4	0.8	0.4	1	0.1414	0.2828	35.4%	11.1%	16	20
3338		4	0.6	0.6	0.6	0	0	0.0%	33.3%	12	20
8382		4	0	0	0	0	0		100.0%	0	20

~~7d~~ 10 d Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.8	1	1	0.8
190		1	0.6	0.6	1
517		1	1	0.6	1
1346		0.8	1	1	0.4
3338		0.6	0.6	0.6	0.6
8382		0	0	0	0

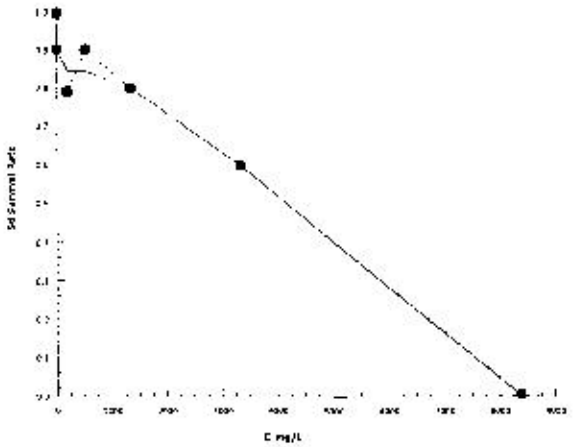
~~7d~~ 10 d Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	4/5	5/5	5/5	4/5
190		4/4	3/5	3/5	5/5
517		5/5	5/5	3/5	5/5
1346		4/5	5/5	5/5	2/5
3338		3/5	3/5	3/5	3/5
8382		0/5	0/5	0/5	0/5

DMa 4/24/16 E

Analysis ID:	16-0451-4819	Endpoint:	~~5d~~ 7(0 Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:15	Analysis:	Trimmed Spearman-Karber	Official Results:	Yes

Graphics



CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 14:16 (p 1 of 2)
Test Code: 468-010-70limb | 33-1680-6292

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	06-7739-8150	Endpoint:	~~6d Survival~~ Rate (Front Limb Emergence)	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:16	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival (7d)	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~224~~

Sample ID:	09-4549-8235	Code:	385B287B	Client:	Confidential
Sample Date:	~~20 May-15~~	Material:	~~Not Applicable~~ NTC	Project:	Special Studies
Receive Date:	~~26 May-15 09:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	36.3%	3338	8382	5290	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	0.4624	2.36	0.316	6	0.6209	CDF	Non-Significant Effect
0.04		517	0.8554	2.36	0.316	6	0.4455	CDF	Non-Significant Effect
0.04		1346	0.3751	2.36	0.316	6	0.6585	CDF	Non-Significant Effect
0.04		3338	2.054	2.36	0.316	6	0.0851	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1799415	0.04498538	4	1.249	0.3328	Non-Significant Effect
Error	0.5403445	0.03602297	15			
Total	0.7202861		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.094	13.3	0.5423	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9577	0.866	0.4965	Normal Distribution

~~6d Survival~~ Rate Summary : Front Limb Emergence

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	0.8	0.5402	1	0.8	0.6	1	0.08165	20.4%	0.0%
190		4	0.75	0.4453	1	0.7	0.6	1	0.09574	25.5%	6.25%
517		4	0.7	0.6163	0.8837	0.7	0.6	0.8	0.05774	16.5%	12.5%
1346		4	0.75	0.3496	1	0.8	0.4	1	0.1258	33.6%	6.25%
3338		4	0.55	0.3909	0.7091	0.6	0.4	0.6	0.05	18.2%	31.3%
8382		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	1.111	0.813	1.41	1.107	0.8861	1.345	0.09377	16.9%	0.0%
190		4	1.049	0.7195	1.379	0.9966	0.8861	1.318	0.1036	19.8%	5.58%
517		4	0.9966	0.7935	1.2	0.9966	0.8861	1.107	0.06382	12.8%	10.3%
1346		4	1.061	0.6237	1.498	1.107	0.6847	1.345	0.1374	25.9%	4.53%
3338		4	0.8357	0.6755	0.9959	0.8861	0.6847	0.8861	0.05034	12.0%	24.8%
8382		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	79.7%

Form 21-wi/16 E

Lithobates pipiens 70-day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	06-7739-8160	Endpoint:	~~8d Survival~~ Rate 64 Front Limb	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:16	Analysis:	Parametric-Control vs Treatments Emergence	Official Results:	Yes

~~8d Survival~~ Rate Detail : Front Limb Emergence

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.6	1	0.8	0.8
190		1	0.6	0.6	0.8
517		0.6	0.8	0.6	0.8
1346		0.8	0.8	1	0.4
3338		0.4	0.6	0.6	0.6
8382		0	0	0	0

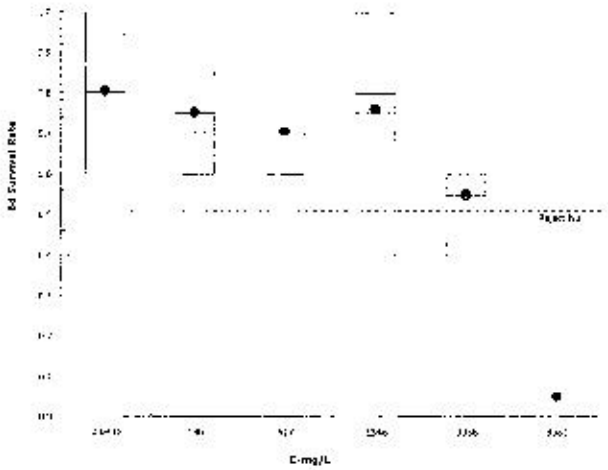
Angular (Corrected) Transformed Detail

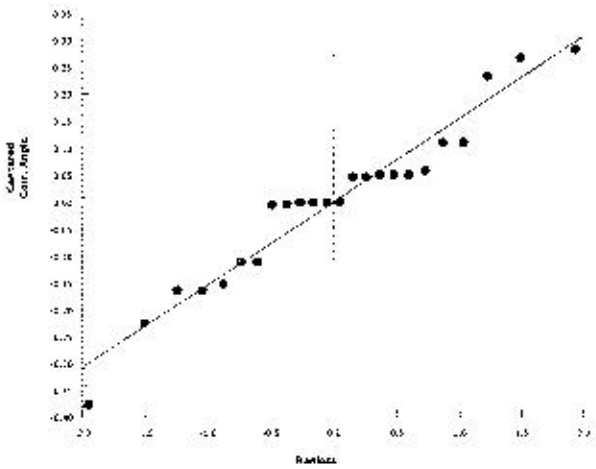
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.8861	1.345	1.107	1.107
190		1.318	0.8861	0.8861	1.107
517		0.8861	1.107	0.8861	1.107
1346		1.107	1.107	1.345	0.6847
3338		0.6847	0.8861	0.8861	0.8861
8382		0.2255	0.2255	0.2255	0.2255

~~8d Survival~~ Rate Binomials : Front Limb Emergence

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	3/5	5/5	4/5	4/5
190		4/4	3/5	3/5	4/5
517		3/5	4/5	3/5	4/5
1346		4/5	4/5	5/5	2/5
3338		2/5	3/5	3/5	3/5
8382		0/5	0/5	0/5	0/5

Graphics





DAP 2/29/16 E

CETIS Analytical Report

Lithobates pipiens 70-Day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date: 24 Feb-16 14:16 (p 1 of 2)
Test Code: 468-010-70limb | 03-1580-6292

TRE Environmental Strategies, LLC

Analysis ID:	01-0679-8463	Endpoint:	~~6d Survival~~ Rate of Front Limb Emergence	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:15	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival 70-Day	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~394~~

Sample ID:	09-4549-8235	Code:	385B287B	Client:	Confidential
Sample Date:	~~20 May-15~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~20 May-15 09:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	676791	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	27.24	N/A	2623
LC10	1381	N/A	2599
LC15	1700	N/A	3811
LC20	2092	N/A	4288
LC25	2575	N/A	4313
LC40	3753	1742	4661
LC50	4291	3047	5146

~~6d Survival~~ Rate Summary Front Limb Emergence — Calculated Variate(A/B)

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0.04	Dilution Water	4	0.8	0.6	1	0.08165	0.1633	20.4%	0.0%	16	20
190		4	0.75	0.6	1	0.09574	0.1915	25.5%	6.25%	14	19
517		4	0.7	0.6	0.8	0.05774	0.1155	16.5%	12.5%	14	20
1346		4	0.75	0.4	1	0.1258	0.2517	33.5%	6.25%	15	20
3338		4	0.55	0.4	0.6	0.05	0.1	18.2%	31.3%	11	20
8382		4	0	0	0	0	0		100.0%	0	20

~~6d Survival~~ Rate Detail Front Limb Emergence

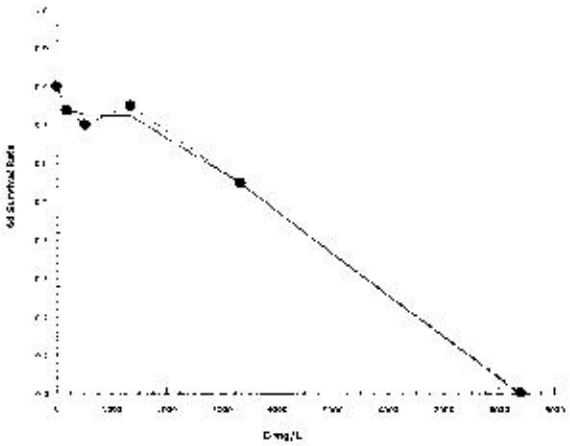
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.6	1	0.8	0.8
190		1	0.6	0.6	0.8
517		0.6	0.8	0.6	0.8
1346		0.8	0.8	1	0.4
3338		0.4	0.6	0.6	0.6
8382		0	0	0	0

~~6d Survival~~ Rate Binomials Front Limb Emergence

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	3/5	5/5	4/5	4/5
190		4/4	3/5	3/5	4/5
517		3/5	4/5	3/5	4/5
1346		4/5	4/5	5/5	2/5
3338		2/5	3/5	3/5	3/5
8382		0/5	0/5	0/5	0/5

Analysis ID:	01-0679-8463	Endpoint: ~~7-d Survival~~ Rate of Trout Limb Emergence		CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:16	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Graphics



Form 42 Rev E

CETIS Analytical Report

Lithobates pipiens 70-D04

~~Fathead Minnow 7 d~~ Larval Survival and Growth Test

Report Date: 24 Feb-16 14:17 (p 1 of 2)
Test Code: 468-010-70limb | 03-1580-6292

TRE Environmental Strategies, LLC

Analysis ID:	06-6419-8245	Endpoint:	~~7d Survival~~ Rate of Complete metamorphosis	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:17	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jun-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~224~~

Sample ID:	09-4549-8235	Code:	3858287B	Client:	Confidential
Sample Date:	~~20 May-15~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~20 May-15 09:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	39.2%	3338	8382	5290	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	1.163	2.36	0.34	6	0.3175	CDF	Non-Significant Effect
0.04		517	1.18	2.36	0.34	6	0.3112	CDF	Non-Significant Effect
0.04		1346	0.3492	2.36	0.34	6	0.6693	CDF	Non-Significant Effect
0.04		3338	2.262	2.36	0.34	6	0.0592	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2554838	0.06387095	4	1.537	0.2419	Non-Significant Effect
Error	0.6232699	0.04155133	15			
Total	0.8787537		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.642	13.3	0.4566	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9731	0.866	0.8192	Normal Distribution

~~7d Survival~~ Rate Summary of Complete metamorphosis

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	0.8	0.5402	1	0.8	0.6	1	0.08165	20.4%	0.0%
190		4	0.65	0.2496	1	0.6	0.4	1	0.1258	38.7%	18.7%
517		4	0.65	0.4909	0.8091	0.6	0.6	0.8	0.05	15.4%	18.7%
1346		4	0.75	0.3496	1	0.8	0.4	1	0.1258	33.6%	6.25%
3338		4	0.5	0.3163	0.6837	0.5	0.4	0.6	0.05774	23.1%	37.5%
8382		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	1.111	0.813	1.41	1.107	0.8861	1.345	0.09377	16.9%	0.0%
190		4	0.9437	0.5189	1.369	0.8861	0.6847	1.318	0.1335	28.3%	15.1%
517		4	0.9413	0.7655	1.117	0.8861	0.8861	1.107	0.05527	11.7%	15.3%
1346		4	1.061	0.6237	1.498	1.107	0.6847	1.345	0.1374	25.9%	4.53%
3338		4	0.7854	0.6004	0.9704	0.7854	0.6847	0.8861	0.05813	14.8%	29.3%
8382		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	79.7%

CETIS Analytical Report

Lithobates pipiens 10-Day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Report Date:	24 Feb-16 14:17 (p 2 of 2)
Test Code:	458-010-70lmb / 03-1660-6292
	TRE Environmental Strategies, LLC

Analysis ID:	08-6419-8245	Endpoint:	~~7d Survival~~ Rate of Complete Metamorphosis	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:17	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

~~7d Survival~~ Rate Detail of Complete metamorphosis

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.6	1	0.8	0.8
190		1	0.6	0.4	0.6
517		0.6	0.6	0.6	0.8
1346		0.8	0.8	1	0.4
3338		0.4	0.6	0.6	0.4
8382		0	0	0	0

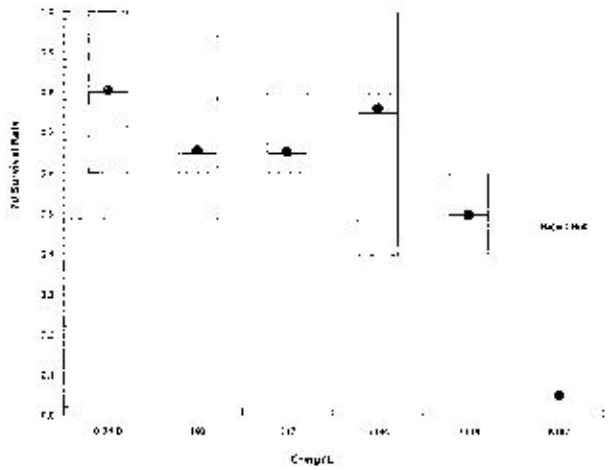
Angular (Corrected) Transformed Detail

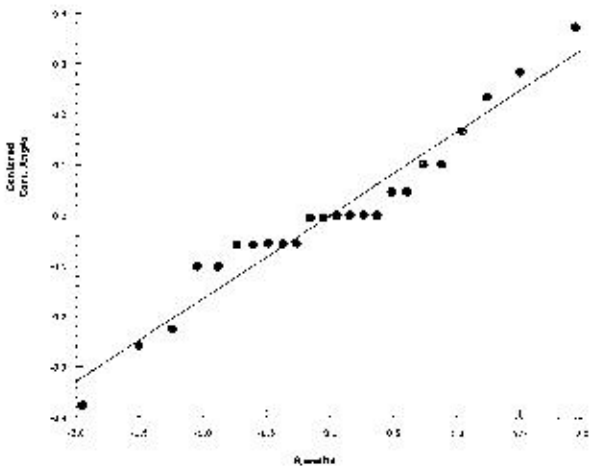
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.8861	1.345	1.107	1.107
190		1.318	0.8861	0.6847	0.8861
517		0.8861	0.8861	0.8861	1.107
1346		1.107	1.107	1.345	0.6847
3338		0.6847	0.8861	0.8861	0.6847
8382		0.2255	0.2255	0.2255	0.2255

~~7d Survival~~ Rate Binomials of Complete metamorphosis

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	3/5	5/5	4/5	4/5
190		4/4	3/5	2/5	3/5
517		3/5	3/5	3/5	4/5
1346		4/5	4/5	5/5	2/5
3338		2/5	3/5	3/5	2/5
8382		0/5	0/5	0/5	0/5

Graphics





Dam 2/24/16 E

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 14:17 (p 1 of 2)
Test Code: 468-010-70limb | 03-1680-6292

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	02-7861-1972	**Endpoint:**	~~7d Survival~~ Rate of Complete Metamorphosis	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 14:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	06-7170-1002	**Test Type:**	Growth-Survival ~~7d~~ 70 d	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	~~224~~

Sample ID:	09-4549-8235	**Code:**	385B287B	**Client:**	Confidential
Sample Date:	~~20 May-15~~	**Material:**	~~Not Applicable~~ WTC	**Project:**	Special Studies
Receive Date:	~~20 May-15 09:10~~	**Source:**	Research		
Sample Age:	~~12h~~	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1377049	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	4.681	N/A	2522
LC10	30.04	N/A	3079
LC15	168.5	N/A	3740
LC20	1629	N/A	4050
LC25	1999	N/A	4369
LC40	3463	504.7	4561
LC50	4013	2619	5057

~~7d Survival~~ Rate Summary of Complete Metamorphosis — Calculated Variate(A/B)

C-mg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0.04	Dilution Water	4	0.8	0.6	1	0.08165	0.1633	20.4%	0.0%	16	20
190		4	0.65	0.4	1	0.1258	0.2517	38.7%	18.7%	12	19
517		4	0.55	0.6	0.8	0.05	0.1	15.4%	18.7%	13	20
1346		4	0.75	0.4	1	0.1258	0.2517	33.6%	6.25%	15	20
3338		4	0.5	0.4	0.6	0.05774	0.1155	23.1%	37.5%	10	20
8382		4	0	0	0	0	0		100.0%	0	20

~~7d Survival~~ Rate Detail of Complete Metamorphosis

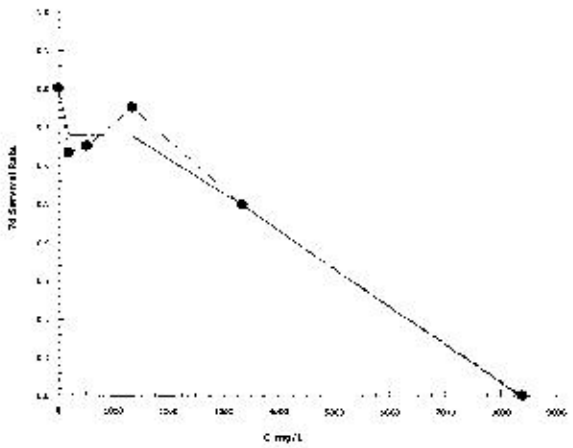
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	0.6	1	0.8	0.8
190		1	0.6	0.4	0.6
517		0.6	0.6	0.6	0.8
1346		0.8	0.8	1	0.4
3338		0.4	0.6	0.6	0.4
8382		0	0	0	0

~~7d Survival~~ Rate Binomials of Complete Metamorphosis

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	3/5	5/5	4/5	4/5
190		4/4	3/5	2/5	3/5
517		3/5	3/5	3/5	4/5
1346		4/5	4/5	5/5	2/5
3338		2/5	3/5	3/5	2/5
8382		0/5	0/5	0/5	0/5

Analysis ID:	02-7861-1972	Endpoint:	~~7d Survival~~ Rate of complete metamorphosis	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:17	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Graphics



CETIS Analytical Report

Lithobates pipiens 10-Day

Report Date: 24 Feb-16 14:18 (p 1 of 2)
Test Code: 468-010-70limb | 03-1580-6292

~~Fathead Minnow 7 d~~ Larval Survival and Growth Test — Blot

TRE Environmental Strategies, LLC

Analysis ID:	13-2310-1471	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:18	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 10d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates p. pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<24~~

Sample ID:	09-4549-8235	Code:	385B287B	Client:	Confidential
Sample Date:	~~20 May-15~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~20 May-15 09:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	35.9%	3338	8382	5290	

Hypothesis = Control < NTO Treatments

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-1.227	2.36	490.4	6	0.9860	CDF	Non-Significant Effect
0.04		517	-0.4794	2.36	490.4	6	0.9178	CDF	Non-Significant Effect
0.04		1346	-0.2806	2.36	490.4	6	0.8779	CDF	Non-Significant Effect
0.04		3338	-1.706	2.36	490.4	6	0.9962	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	345939.8	86484.96	4	0.9981	0.4389	Non-Significant Effect
Error	1299714	86647.6	15			
Total	1645654		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.877	13.3	0.0425	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9832	0.866	0.9685	Normal Distribution

Blot

Mean Dry Biomass-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	1367	911.2	1823	1455	951	1607	143.2	21.0%	0.0%
190		4	1112	352.2	1871	1054	641.6	1698	238.6	42.9%	18.7%
517		4	1267	1200	1334	1256	1231	1327	21.01	3.32%	7.3%
1346		4	1309	878.5	1739	1275	1027	1657	135.2	20.7%	4.27%
3338		4	1012	661.4	1363	1018	784.2	1228	110.2	21.8%	26.0%
8382		4	0	0	0	0	0	0	0		100.0%

Blot

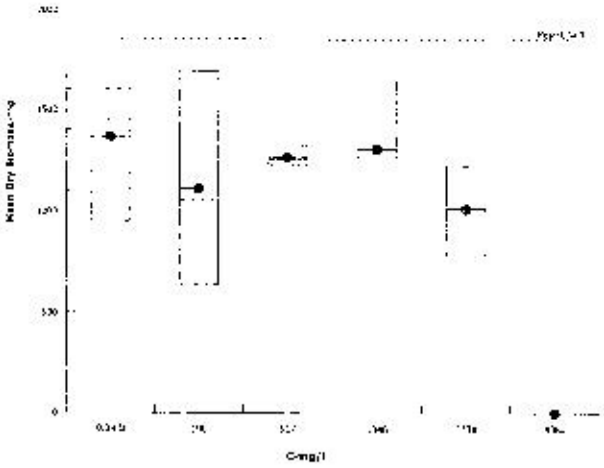
Mean Dry Biomass-mg Detail

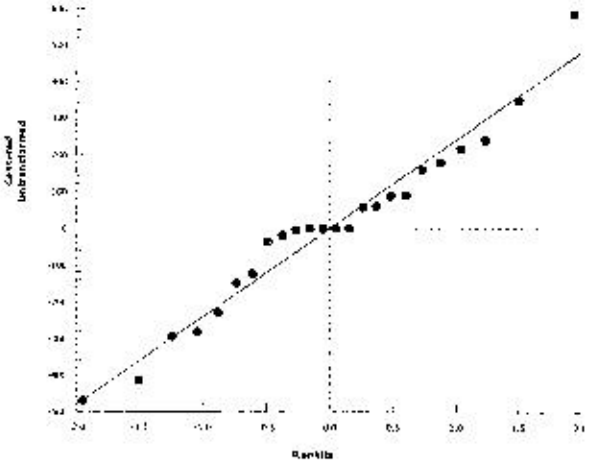
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	951	1607	1455	1454
190		1698	816.7	641.6	1291
517		1327	1263	1231	1248
1346		1185	1365	1657	1027
3338		784.2	1228	1171	864.6
8382		0	0	0	0

Note: These are weights (mg) per original number of frogs.

Analysis ID:	13-2310-1471	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-15 14:18	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





DAMC 2/24/15 E

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 14:18 (p 1 of 2)
Test Code: 468-010-70limb 03-1680-6292

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — TRE Environmental Strategies, LLC

Analysis ID:	03-0801-5012	Endpoint:	Mean Dry Weight-mg	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 14:18	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival (7d) 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~224~~

Sample ID:	09-4549 8235	Code:	3858287B	Client:	Confidential
Sample Date:	~~29 May 15~~	Material:	~~Not Applicable~~ NTC	Project:	Special Studies
Receive Date:	~~29 May 15 09:10~~	Source:	Research		
Sample Age:	~~12h~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	29.4%	3338	>3338	NA	

Hypothesis: Control < NTC treatments

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-0.01856	2.36	502.7	6	0.8059	CDF	Non-Significant Effect
0.04		517	1.287	2.36	502.7	6	0.2719	CDF	Non-Significant Effect
0.04		1346	0.6831	2.36	502.7	6	0.5225	CDF	Non-Significant Effect
0.04		3338	1.513	2.36	502.7	6	0.1999	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	366366.8	91591.7	4	1.006	0.4351	Non-Significant Effect
Error	1365515	91034.33	15			
Total	1731882		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.687	13.3	0.1037	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9375	0.866	0.2146	Normal Distribution

Mean Dry Weight-mg Summary

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	1707	1502	1913	1712	1585	1820	64.52	7.56%	0.0%
190		4	1703	1178	2229	1651	1361	2151	165.2	19.4%	0.23%
517		4	1982	1522	2442	2078	1560	2212	144.7	14.6%	-16.1%
1346		4	1853	1080	2626	1682	1481	2567	243	26.2%	-8.54%
3338		4	2030	1875	2186	2004	1951	2162	48.83	4.81%	-18.9%

Mean Dry Weight-mg Detail

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	1585	1607	1820	1818
190		1698	1361	1604	2151
517		2212	2106	2051	1560
1346		1481	1707	1657	2567
3338		1961	2047	1951	2162

Note: These are weight (mg) per surviving number of frogs

Dam 4/24/16

Lithodates pipiens 70-Day

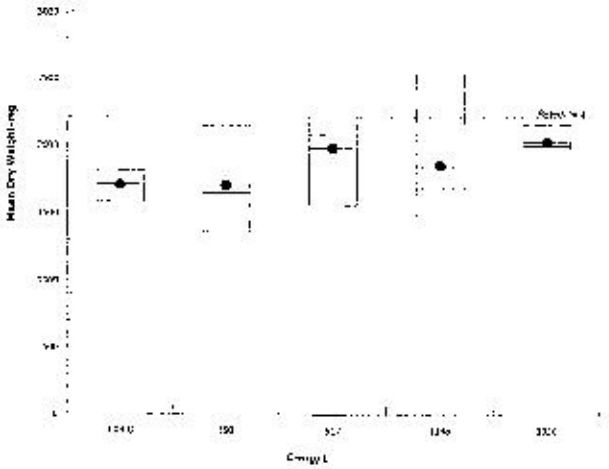
~~Pathead Minnow 7-d~~ Larval Survival and Growth Test

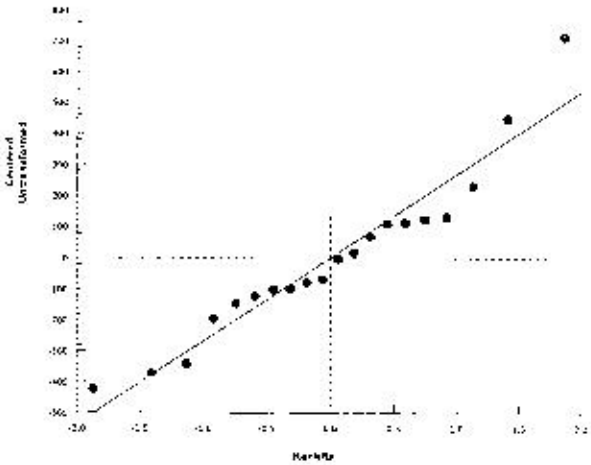
Biot

TRE Environmental Strategies, LLC

Analysis ID:	03-0801-5012	**Endpoint:**	Mean Dry Weight-mg	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 14:18	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





DMM 2/24/16 E

Snout to Vent Length

CETIS Analytical Report

(Body Length)

Lithobates pipiens 70-d

Report Date: 24 Feb-16 15:03 (p 1 of 2)
Test Code: 468-010length 16-7039-1907

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test SVL-mm

TRE Environmental Strategies, LLC

Analysis ID:	03-2802-5301	**Endpoint:**	~~Mean Dry Weight mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:03	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	06-7170-1002	**Test Type:**	Growth-Survival ~~(7d)~~ 70d	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	~~<24h~~

Sample ID:	14-9337 7493	**Code:**	590321D5	**Client:**	Confidential
Sample Date:	27 Oct-15 10:47	**Material:**	~~Not Applicable~~ WTC	**Project:**	Special Studies
Receive Date:	~~27 Oct-15 10:47~~	**Source:**	Research		
Sample Age:	~~NA~~	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	10.5%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	1.244	2.36	2.703	6	0.2674	CDF	Non-Significant Effect
0.04		517	-0.6976	2.36	2.703	6	0.9489	CDF	Non-Significant Effect
0.04		1346	-0.425	2.36	2.703	6	0.9081	CDF	Non-Significant Effect
0.04		3338	-1.254	2.36	2.703	6	0.9870	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	18.58471	4.646177	4	1.766	0.1883	Non-Significant Effect
Error	39.4702	2.631347	15			
Total	58.05491		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.443	13.3	0.0509	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9669	0.866	0.6888	Normal Distribution

~~Mean Dry Weight mg~~ Summary of SVL-mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	25.85	25.23	26.48	25.92	25.37	26.2	0.1969	1.52%	0.0%
190		4	24.43	20.74	28.12	25.23	21.07	26.18	1.16	9.49%	5.52%
517		4	26.66	24.32	28.99	26.84	24.74	28.2	0.7326	5.5%	-3.09%
1346		4	26.34	22.75	29.93	25.77	24.29	29.55	1.128	8.56%	-1.89%
3338		4	27.29	26.3	28.29	27.16	26.7	28.15	0.3126	2.29%	-5.56%

~~Mean Dry Weight mg~~ Detail of SVL-mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	26.2	25.37	26.15	25.7
190		25.71	21.07	24.75	26.18
517		27.23	26.45	28.2	24.74
1346		24.29	25.56	25.97	29.55
3338		28.15	26.7	27	27.33

Snout to Vent Length

CETIS Analytical Report

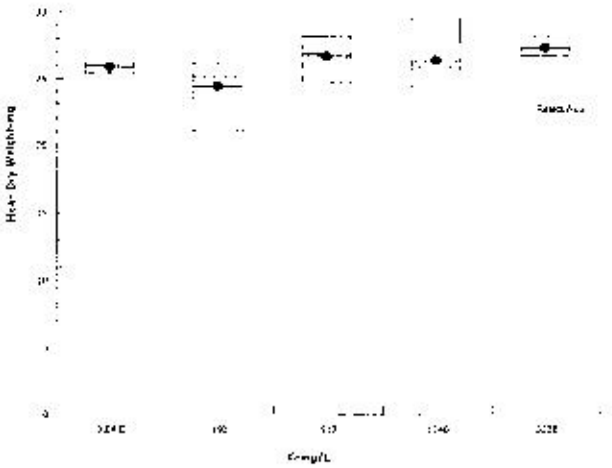
Lithobates pipiens 70-Day

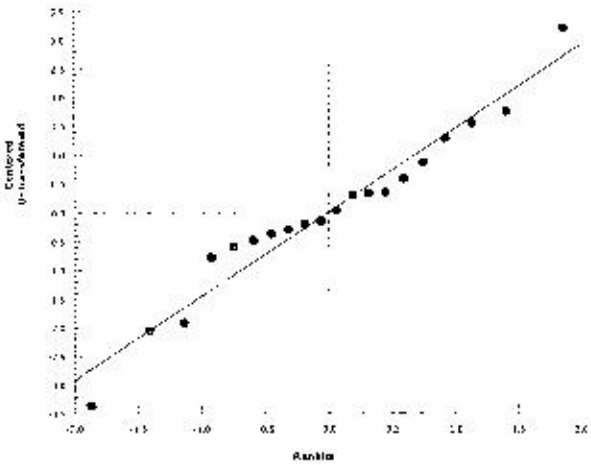
		Report Date:	24 Feb-16 15:03 (p 2 of 2)
		Test Code:	468-010length | 16-7039-1907

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Sub-mm TRE Environmental Strategies, LLC

Analysis ID:	03-2802-5301	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:03	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





Esmc 4/21/16 E

Snout to Vent Length

CETIS Analytical Report

Lithobates Pipiens 10-day

Report Date: 24 Feb-16 15:04 (p 1 of 2)
Test Code: 468-010ength : 16-7039-1907

~~Pathead Minnow 7-d~~ Larval Survival and Growth Test SVL-mm

TRE Environmental Strategies, LLC

Analysis ID:	11-9507-0525	**Endpoint:**	~~Mean Dry Weight-mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	06-7170-1002	**Test Type:**	Growth-Survival ~~(7d)~~ 10 d	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates Pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	~~<24~~

Sample ID:	14-9337-7493	**Code:**	590321D5	**Client:**	Confidential
Sample Date:	~~27 Oct-15 10:47~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~27 Oct-15 10:47~~	**Source:**	Research		
Sample Age:	~~NA~~	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	10.5%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-1.244	2.36	2.703	6	0.9866	CDF	Non-Significant Effect
0.04		517	0.6976	2.36	2.703	6	0.5160	CDF	Non-Significant Effect
0.04		1346	0.425	2.36	2.703	6	0.6372	CDF	Non-Significant Effect
0.04		3338	1.254	2.36	2.703	6	0.2837	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	18.58471	4.646177	4	1.766	0.1883	Non-Significant Effect
Error	39.4702	2.631347	15			
Total	58.05491		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.443	13.3	0.0509	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9669	0.866	0.6888	Normal Distribution

~~Mean Dry Weight-mg~~ Summary of SVL-mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	25.85	25.23	26.48	25.92	25.37	26.2	0.1969	1.52%	0.0%
190		4	24.43	20.74	28.12	25.23	21.07	26.18	1.16	9.49%	5.52%
517		4	26.66	24.32	28.99	26.84	24.74	28.2	0.7326	5.5%	-3.09%
1346		4	26.34	22.75	29.93	25.77	24.29	29.55	1.128	8.56%	-1.89%
3338		4	27.29	26.3	28.29	27.16	25.7	28.15	0.3126	2.29%	-5.56%

~~Mean Dry Weight-mg~~ Detail of SVL-mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	26.2	25.37	26.15	25.7
190		25.71	21.07	24.75	26.18
517		27.23	26.45	28.2	24.74
1346		24.29	25.56	25.97	29.55
3338		28.15	26.7	27	27.33

DMT 2/24/16 E

Snout to Vent Length

CETIS Analytical Report

Lithobates Pipiens 70-Day

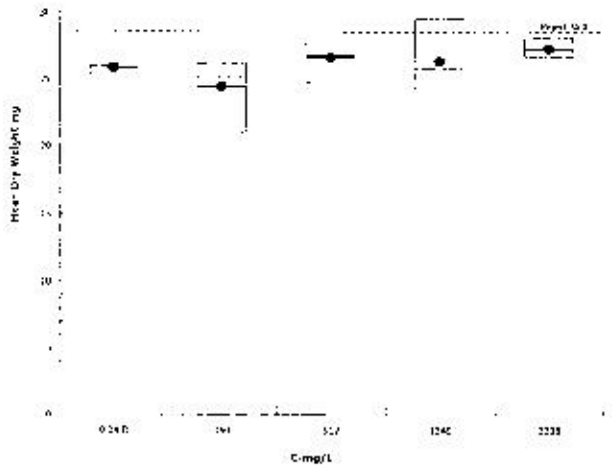
Report Date: 24 Feb-16 15:04 (p 2 of 2)
Test Code: 468-010length | 16-7039-1907

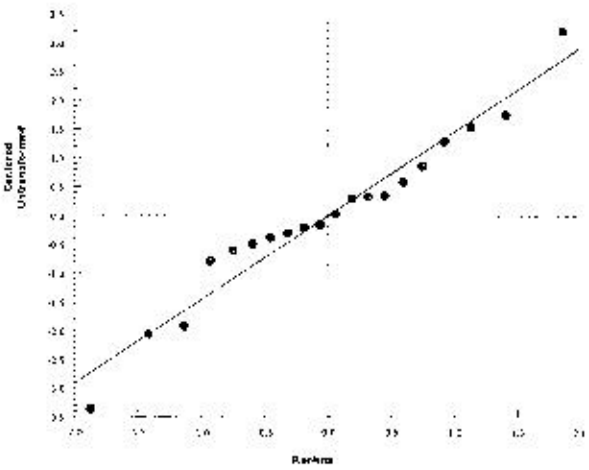
~~Fathead Minnow 7 d~~ Larval Survival and Growth Test SVL-mm

TRE Environmental Strategies, LLC

Analysis ID:	11-9507-0525	**Endpoint:**	~~Mean Dry Weight-mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Right Radio - Ulna

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 15:05 (p 1 of 2)
Test Code: 466-010RtRU | 16-2683-0301

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

TRE Environmental Strategies, LLC

Analysis ID:	07-5829-6311	Endpoint:	~~Mean Dry Weight mg~~ Length - mm	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:05	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~234~~

Sample ID:	04-6797-3948	Code:	1BE4873C	Client:	Confidential
Sample Date:	~~27 Oct 15 11:13~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27 Oct 15 11:13~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	12.3%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190*	2.59	2.36	0.724	6	0.0325	CDF	Significant Effect
0.04		517	-0.2312	2.36	0.724	6	0.8661	CDF	Non-Significant Effect
0.04		1346	-0.1058	2.36	0.724	6	0.8324	CDF	Non-Significant Effect
0.04		3338	-0.16	2.36	0.724	6	0.8476	CDF	Non-Significant Effect

ANOVA Table

Higher NOEL Chosen

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.235485	0.5588713	4	2.96	0.0548	Non-Significant Effect
Error	2.831774	0.188785	15			
Total	5.06726		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.322	13.3	0.1198	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9754	0.866	0.8625	Normal Distribution

~~Mean Dry Weight mg~~ Summary : Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	5.874	5.584	6.164	5.91	5.65	6.025	0.09118	3.1%	0.0%
190		4	5.076	3.867	6.289	5.131	4.3	5.75	0.3806	15.0%	13.6%
517		4	5.945	5.573	6.317	5.942	5.662	6.233	0.117	3.94%	-1.21%
1346		4	5.906	5.181	6.631	5.838	5.45	6.5	0.2278	7.72%	-0.55%
3338		4	5.923	5.505	6.34	5.863	5.65	6.275	0.1312	4.43%	-0.84%

~~Mean Dry Weight mg~~ Detail : Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	5.8	6.02	6.025	5.65
190		5.713	4.55	4.3	5.75
517		6.233	5.967	5.917	5.662
1346		5.675	6	5.45	6.5
3338		6.275	5.933	5.833	5.65

QAm 4/29/16 E

Right Radio-Ulna

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 15:05 (p 2 of 2)
Test Code: 468-010R1RU | 16-2683-0301

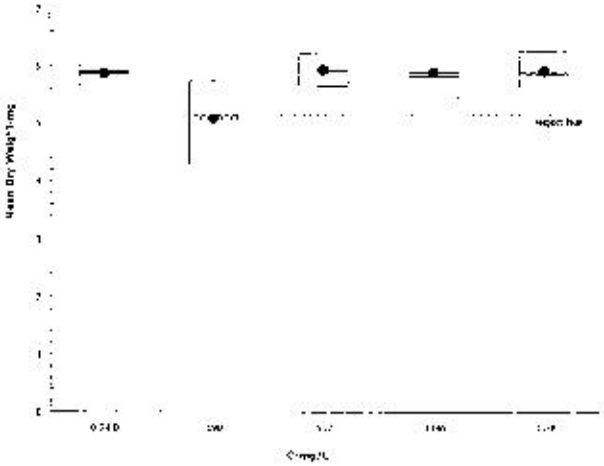
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

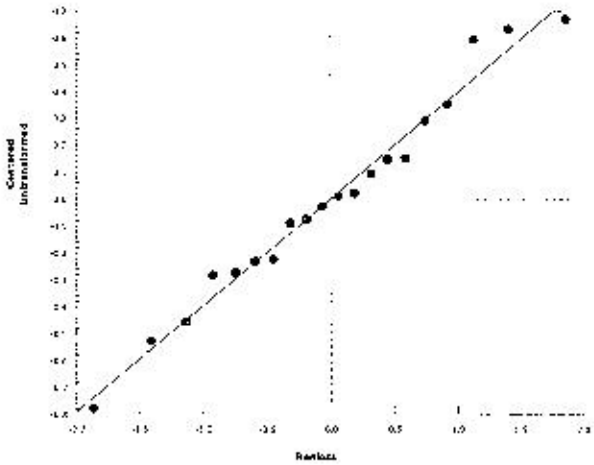
length-mm

TRE Environmental Strategies, LLC

Analysis ID:	07-5829-6311	Endpoint:	~~Mean Dry Weight mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:05	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





Ⓒ AMR 2/24/16 E

CETIS Analytical Report

Right Radio-Ulna

Lithobates Pipiens 70-Day

Report Date:	24 Feb-16 15:05 (p 1 of 2)		
Test Code:	468-010RtRU | 18-2383-0301		

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — Length - mm

TRE Environmental Strategies, LLC

Analysis ID:	18-2365-8366	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:05	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(14)~~ 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates Pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~224~~

Sample ID:	04-6797-3948	Code:	18E4B73C	Client:	Confidential
Sample Date:	~~27 Oct-15 11:13~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27 Oct-15 11:18~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	12.3%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-2.59	2.36	0.724	6	0.9997	CDF	Non-Significant Effect
0.04		517	0.2312	2.36	0.724	6	0.7171	CDF	Non-Significant Effect
0.04		1346	0.1058	2.36	0.724	6	0.7640	CDF	Non-Significant Effect
0.04		3338	0.16	2.36	0.724	6	0.7443	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.235485	0.5588713	4	2.96	0.0548	Non-Significant Effect
Error	2.831774	0.188785	15			
Total	5.06726		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.322	13.3	0.1198	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9754	0.866	0.8625	Normal Distribution

~~Mean Dry Weight-mg~~ Summary : Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	5.874	5.584	6.164	5.91	5.65	6.025	0.09118	3.1%	0.0%
190		4	6.078	3.867	8.289	5.131	4.3	5.75	0.3806	15.0%	-13.5%
517		4	5.945	5.573	6.317	5.942	5.662	6.233	0.117	3.94%	-1.21%
1346		4	5.906	5.181	6.631	5.838	5.45	6.5	0.2278	7.72%	-0.55%
3338		4	5.923	5.505	6.34	5.883	5.65	6.275	0.1312	4.43%	-0.84%

~~Mean Dry Weight-mg~~ Detail Length - mm

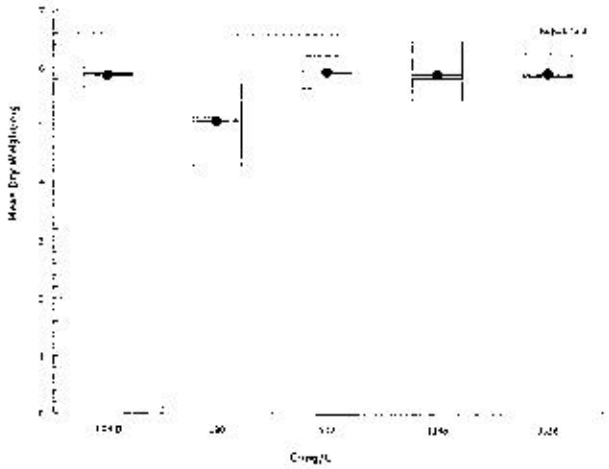
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	5.8	6.02	6.025	5.65
190		5.713	4.55	4.3	5.75
517		6.233	5.967	5.917	5.662
1346		5.675	6	5.45	6.5
3338		6.275	5.933	5.833	5.65

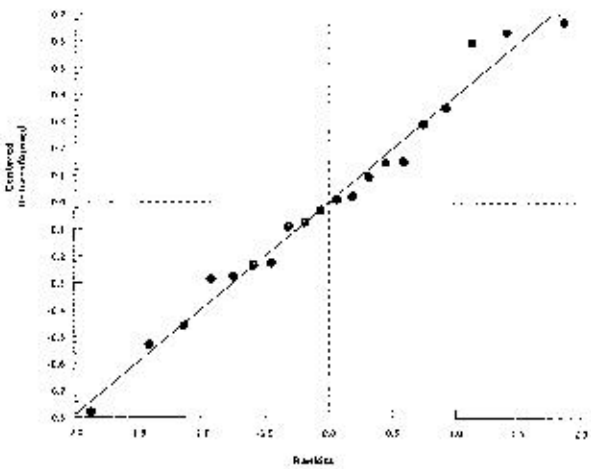
Arm 4/24/16 E

Right Ratio - Ultra
Lithobates pipiens 10-Day
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test
Length - mm
TRE Environmental Strategies, LLC

Analysis ID:	18-2365-8366	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:05	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





a 5m 2/24/16 E

CETIS Analytical Report

Left to Right - Ulna

Lithobates Pipiens 10-Day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Length - mm

Report Date:	24 Feb-16 15:06 (p 1 of 2)		
Test Code:	468-010LfRU | 17-6919-2467		
TRE Environmental Strategies, LLC			

Analysis ID:	01-3099-4885	Endpoint:	Mean Dry Weight-mg	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:06	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~7d~~ 10-Day	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates Pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<24~~

Sample ID:	21-0154-0932	Code:	7D42F844	Client:	Confidential
Sample Date:	~~27 Oct-15 11:20~~	Material:	~~Not Applicable~~ NTC	Project:	Special Studies
Receive Date:	~~27 Oct-15 11:20~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	9.04%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Higher NOEL Chosen

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190*	4.025	2.36	0.531	6	0.0019	CDF	Significant Effect
0.04		517	1.585	2.36	0.531	6	0.1800	CDF	Non-Significant Effect
0.04		1346	1.571	2.36	0.531	6	0.1837	CDF	Non-Significant Effect
0.04		3338	0.7533	2.36	0.531	6	0.4909	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.86129	0.4653225	4	4.58	0.0129	Significant Effect
Error	1.524028	0.1016019	15			
Total	3.385318		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.738	13.3	0.1504	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9677	0.866	0.7052	Normal Distribution

~~Mean Dry Weight-mg~~ Summary Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	5.876	5.519	6.233	5.851	5.667	6.135	0.112	3.81%	0.0%
190		4	4.969	4.108	5.83	4.9	4.425	5.65	0.2705	10.9%	15.4%
517		4	5.519	5.237	5.8	5.525	5.325	5.7	0.08848	3.21%	6.08%
1346		4	5.522	4.975	6.069	5.45	5.238	5.95	0.1719	6.23%	6.03%
3338		4	5.706	5.507	5.905	5.7	5.575	5.85	0.06249	2.19%	2.89%

~~Mean Dry Weight-mg~~ Detail Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	5.667	5.99	6.135	5.713
190		5.65	4.667	4.425	5.133
517		5.7	5.417	5.633	5.325
1346		5.238	5.65	5.25	5.95
3338		5.85	5.633	5.767	5.575

(1) EMM 4-24-16 EF

Left Radio-Ulna

Lithobates pipiens 70-Day

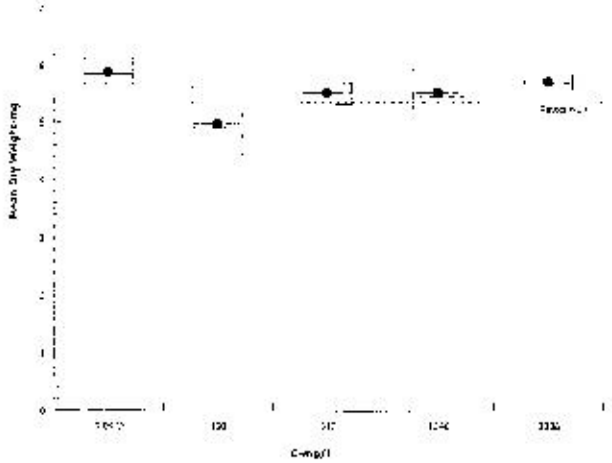
Fathead Minnow 7-d Larval Survival and Growth Test

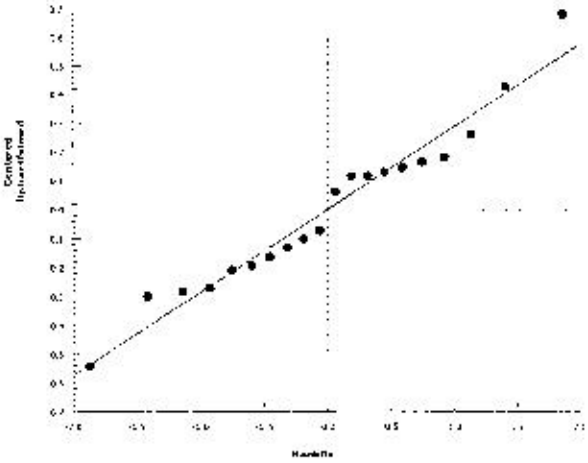
Length-mm

TRE Environmental Strategies, LLC

Analysis ID:	01-3099-4885	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:06	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





① Am 2/24/16 E

Analyst: DMR QA: 02/23/16

Left Ratio - Cina

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date:	24 Feb-16 15:07 (p 1 of 2)
Test Code:	468-010LfRU : 17-6919-2467

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test — Length - mm — TRE Environmental Strategies, LLC

Analysis ID:	14-3423-5233	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:07	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<72h~~

Sample ID:	21-0154-0932	Code:	7D42F944	Client:	Confidential
Sample Date:	~~27 Oct-15 11:20~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27 Oct-15 11:20~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	9.04%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-4.025	2.36	0.531	6	1.0000	CDF	Non-Significant Effect
0.04		517	-1.585	2.36	0.531	6	0.9947	CDF	Non-Significant Effect
0.04		1346	-1.571	2.36	0.531	6	0.9945	CDF	Non-Significant Effect
0.04		3338	-0.7533	2.36	0.531	6	0.9550	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.86129	0.4653225	4	4.58	0.0129	Significant Effect
Error	1.524028	0.1016019	15			
Total	3.385318		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.738	13.3	0.1504	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9677	0.866	0.7052	Normal Distribution

~~Mean Dry Weight-mg~~ Summary : Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	5.876	5.519	6.233	5.851	5.667	6.135	0.112	3.81%	0.0%
190		4	4.969	4.108	5.83	4.9	4.425	5.65	0.2705	10.9%	15.4%
517		4	5.519	5.237	5.8	5.525	5.325	5.7	0.06848	3.21%	6.08%
1346		4	5.522	4.975	6.069	5.45	5.236	5.95	0.1719	6.23%	6.03%
3338		4	5.706	5.507	5.905	5.7	5.575	5.85	0.06249	2.19%	2.89%

~~Mean Dry Weight-mg~~ Detail : Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	5.667	5.99	6.135	5.713
190		5.65	4.667	4.425	5.133
517		5.7	5.417	5.633	5.325
1346		5.236	5.65	5.25	5.95
3338		5.85	5.633	5.767	5.575

DMM 2/24/16 E

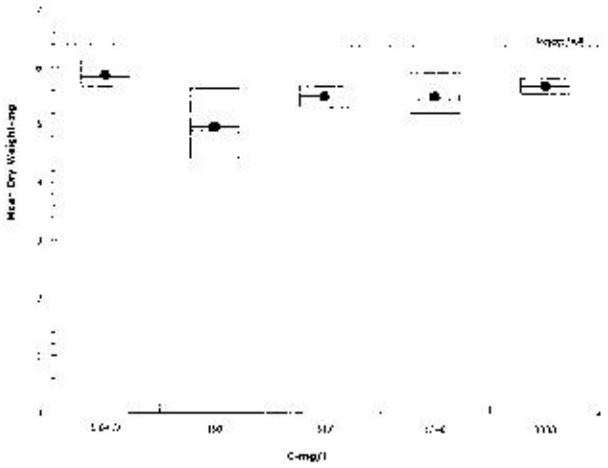
Left Radio-Ulna

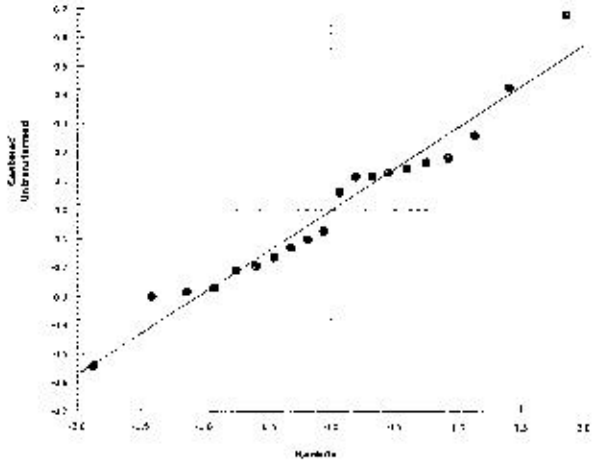
CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date:	24 Feb-16 15:07 (p 2 of 2)		
Test Code:	468-010LfRU | 17-6919-2467		
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test		TRE Environmental Strategies, LLC	
Analysis ID:	14-3423-5233	Endpoint:	~~Mean Dry Weight-mg~~ Length-mm
Analyzed:	24 Feb-16 15:07	Analysis:	Parametric-Control vs Treatments
CETIS Version:	CETISv1.8.7		
Official Results:	Yes		

Graphics





DMC 2) 24 16 E

CETIS Analytical Report

Right Femur

Lithobates pipiens 10-Day

Report Date: 24 Feb-16 15:08 (p 1 of 2)
Test Code: 466-01DRF | 03-2725-2227

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length-mm

TRE Environmental Strategies, LLC

Analysis ID:	18-2502-6202	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:07	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 10 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<24h~~

Sample ID:	06-0350-4209	Code:	23F8BE51	Client:	Confidential
Sample Date:	~~27-Oct-15 11:24~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27-Oct-15 11:24~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	15.9%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	1.595	2.36	1.781	6	0.1773	CDF	Non-Significant Effect
0.04		517	-0.02783	2.36	1.781	6	0.8089	CDF	Non-Significant Effect
0.04		1346	0.6027	2.36	1.781	6	0.5587	CDF	Non-Significant Effect
0.04		3338	0.05346	2.36	1.781	6	0.7822	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.396106	1.099026	4	0.9616	0.4568	Non-Significant Effect
Error	17.14408	1.142938	15			
Total	21.54018		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	12.28	13.3	0.0154	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9598	0.866	0.5402	Normal Distribution

~~Mean Dry Weight-mg~~ Summary : Length-mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	11.21	10.9	11.52	11.24	10.95	11.41	0.09637	1.72%	0.0%
190		4	10.01	8.16	11.86	10.34	8.4	10.94	0.5798	11.6%	10.8%
517		4	11.23	9.245	13.22	11.46	9.563	12.45	0.6245	11.1%	-0.19%
1346		4	10.76	8.149	13.36	10.06	9.712	13.2	0.819	15.2%	4.06%
3338		4	11.17	10.69	11.65	11.13	10.9	11.53	0.1501	2.69%	0.36%

~~Mean Dry Weight-mg~~ Detail : Length-mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	10.95	11.22	11.26	11.41
190		10.94	9.917	8.4	10.77
517		12.45	11.85	11.07	9.563
1346		10.0	9.712	10.01	13.2
3338		11.3	10.95	11.53	10.9

[illegible] 2/24/16 E

Right Femur

Lithobates pipiens 10-Day

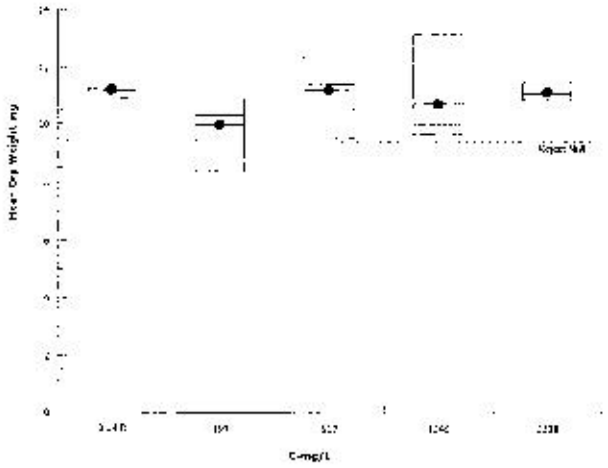
~~Pathead Minnow 7-d~~ Larval Survival and Growth Test

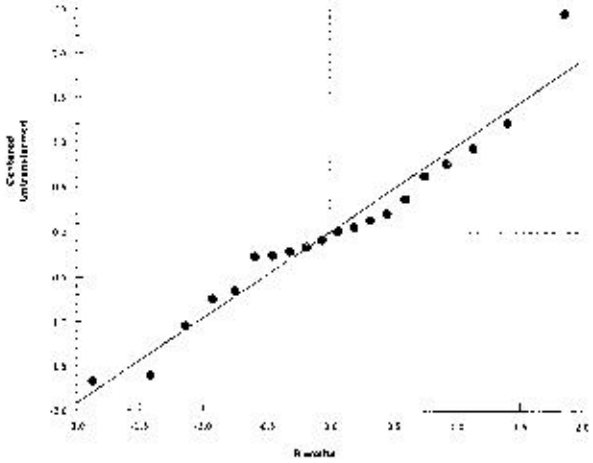
Length - mm

TRE Environmental Strategies, LLC

Analysis ID:	16-2502-6202	Endpoint:	~~Mean Dry Weight mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:07	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





@ RMR 2/24/16 FL

Right Femur

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 15:08 (p 1 of 2)
Test Code: 458-010RF | 03-2725-2227

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length - mm

TRE Environmental Strategies, LLC

Analysis ID:	16-6807-2247	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:08	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<72h~~

Sample ID:	06-0350-4209	Code:	23F8BE51	Client:	Confidential
Sample Date:	~~27 Oct-15 11:24~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27 Oct-15 11:24~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	15.9%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-1.595	2.36	1.781	6	0.9948	CDF	Non-Significant Effect
0.04		517	0.02783	2.36	1.781	6	0.7909	CDF	Non-Significant Effect
0.04		1346	-0.6027	2.36	1.781	6	0.9368	CDF	Non-Significant Effect
0.04		3338	-0.05346	2.36	1.781	6	0.8166	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.396106	1.099026	4	0.9616	0.4568	Non-Significant Effect
Error	17.14408	1.142938	15			
Total	21.54018		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	12.28	13.3	0.0154	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9598	0.866	0.5402	Normal Distribution

~~Mean Dry Weight-mg~~ Summary : Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	11.21	10.9	11.52	11.24	10.95	11.41	0.09637	1.72%	0.0%
190		4	10.01	8.16	11.85	10.34	8.4	10.94	0.5798	11.6%	10.8%
517		4	11.23	9.245	13.22	11.46	9.563	12.45	0.6245	11.1%	-0.19%
1346		4	10.76	8.149	13.36	10.06	9.712	13.2	0.819	15.2%	4.06%
3338		4	11.17	10.69	11.65	11.13	10.9	11.53	0.1501	2.69%	0.36%

~~Mean Dry Weight-mg~~ Detail : Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	10.95	11.22	11.26	11.41
190		10.94	9.917	8.4	10.77
517		12.45	11.85	11.07	9.563
1346		10.1	9.712	10.01	13.2
3338		11.3	10.95	11.53	10.9

Right Femur

CETIS Analytical Report

Report Date: 24 Feb-16 15:08 (p 2 of 2)
Test Code: 458-010RF | 03-2725-2227

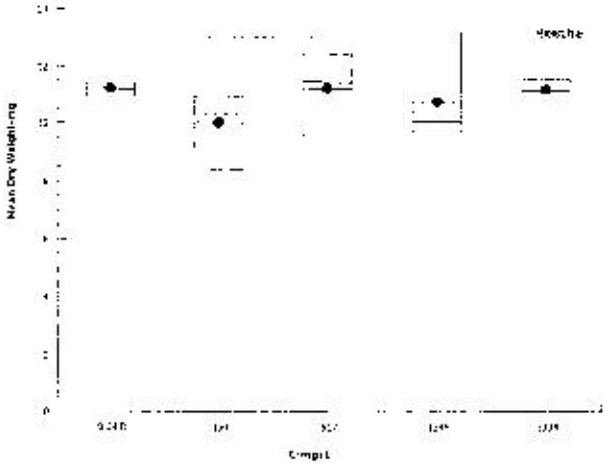
~~Lithospi~~ Lithobates pipiens 70-Day

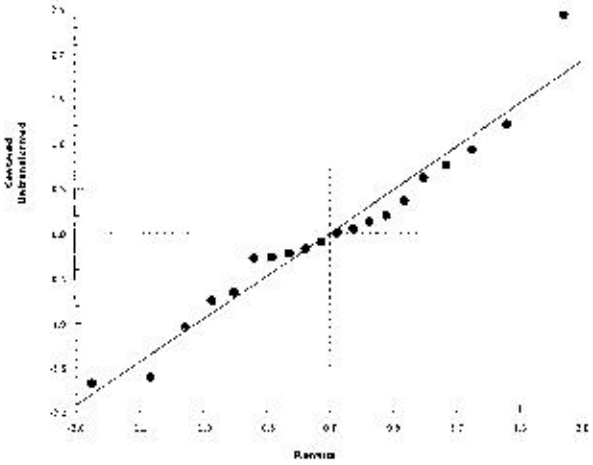
~~Fathead Minnow 7 d~~ Larval Survival and Growth Test Length - mm

TRE Environmental Strategies, LLC

Analysis ID:	16-6807-2247	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:08	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





DAMR 2/24/16 E

CETIS Analytical Report

Left Femur

Lithobates pipiens 70-Day

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Length - mm

Report Date:	24 Feb-16 15:09 (p 1 of 2)
Test Code:	468-010LF ; 17-7859-8882

TRE Environmental Strategies, LLC

Analysis ID:	05-1749-7724	Endpoint:	~~Mean Dry Weight mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:08	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 70d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<24h~~

Sample ID:	11-3969-1826	Code:	43EE5132	Client:	Confidential
Sample Date:	~~27 Oct-15 11:31~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27 Oct-15 11:31~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	16.8%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	1.338	2.36	1.776	6	0.2544	CDF	Non-Significant Effect
0.04		517	-0.1957	2.36	1.776	6	0.8570	CDF	Non-Significant Effect
0.04		1346	0.3231	2.36	1.776	6	0.6802	CDF	Non-Significant Effect
0.04		3338	-0.3809	2.36	1.776	6	0.8995	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.189328	1.047332	4	0.9217	0.4771	Non-Significant Effect
Error	17.04524	1.136349	15			
Total	21.23457		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.62	13.3	0.0312	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9134	0.866	0.0739	Normal Distribution

~~Mean Dry Weight mg~~ Summary : Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	11.27	10.8	11.74	11.28	10.91	11.6	0.1489	2.64%	0.0%
190		4	10.25	7.769	12.75	9.992	8.675	12.38	0.7829	15.3%	8.95%
517		4	11.42	9.989	12.84	11.58	10.2	12.32	0.4485	7.86%	-1.31%
1346		4	11.03	8.639	13.41	10.62	9.738	13.13	0.7499	13.6%	2.16%
3338		4	11.56	11.09	12.03	11.57	11.18	11.9	0.1477	2.55%	-2.55%

~~Mean Dry Weight mg~~ Detail : Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	11.17	10.91	11.6	11.4
190		10.3	9.583	8.675	12.38
517		11.38	12.32	11.77	10.2
1346		10.2	9.738	11.04	13.13
3338		11.52	11.62	11.18	11.9

DMM 2/24/16 E

Left Femur

Lithobates pipiens 7C-Day

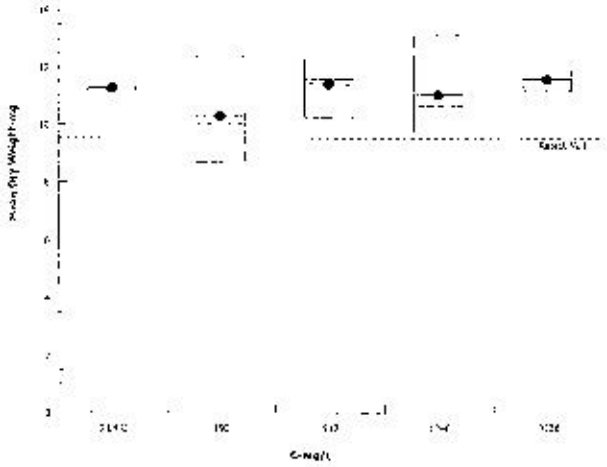
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

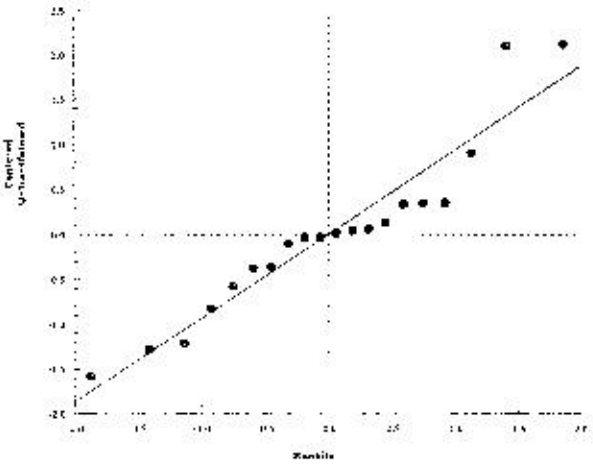
Length-mm

TRE Environmental Strategies, LLC

Analysis ID:	05-1749-7724	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:08	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





Dave Mallot

CETIS Analytical Report

Left Femur

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 15:09 (p 1 of 2)
Test Code: 468-010LF | 17-7859-8882

~~Fathead Minnow 7 d~~ Larval Survival and Growth Test Length - mm

TRE Environmental Strategies, LLC

Analysis ID:	10-2588-2337	**Endpoint:**	~~Mean Dry Weight-mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:09	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	06-7170-1002	**Test Type:**	Growth-Survival ~~(7d)~~ 70-Day	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	68d 19h	**Source:**	Carolina Biological Supply	**Age:**	~~224~~

Sample ID:	11-3969-1826	**Code:**	43EE5132	**Client:**	Confidential
Sample Date:	~~27 Oct-15 11:34~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~27 Oct-15 11:31~~	**Source:**	Research		
Sample Age:	~~NA~~	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	15.8%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-1.338	2.36	1.776	6	0.9895	CDF	Non-Significant Effect
0.04		517	0.1957	2.36	1.776	6	0.7308	CDF	Non-Significant Effect
0.04		1346	-0.3231	2.36	1.776	6	0.8874	CDF	Non-Significant Effect
0.04		3338	0.3809	2.36	1.776	6	0.6560	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.189328	1.047332	4	0.9217	0.4771	Non-Significant Effect
Error	17.04524	1.136349	15			
Total	21.23457		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.62	13.3	0.0312	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9134	0.866	0.0739	Normal Distribution

~~Mean Dry Weight-mg~~ Summary : Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	11.27	10.8	11.74	11.28	10.91	11.6	0.1489	2.64%	0.0%
190		4	10.26	7.769	12.76	9.992	8.675	12.38	0.7829	15.3%	8.95%
517		4	11.42	9.989	12.84	11.58	10.2	12.32	0.4485	7.86%	-1.31%
1346		4	11.03	8.639	13.41	10.62	9.738	13.13	0.7499	13.6%	2.16%
3338		4	11.56	11.09	12.03	11.57	11.18	11.9	0.1477	2.56%	-2.55%

~~Mean Dry Weight-mg~~ Detail : Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	11.17	10.91	11.6	11.4
190		10.3	9.683	8.675	12.38
517		11.38	12.32	11.77	10.2
1346		10.2	9.738	11.04	13.13
3338		11.62	11.62	11.18	11.9

DMM 4-24-16 E

Left Femur

CETIS Analytical Report

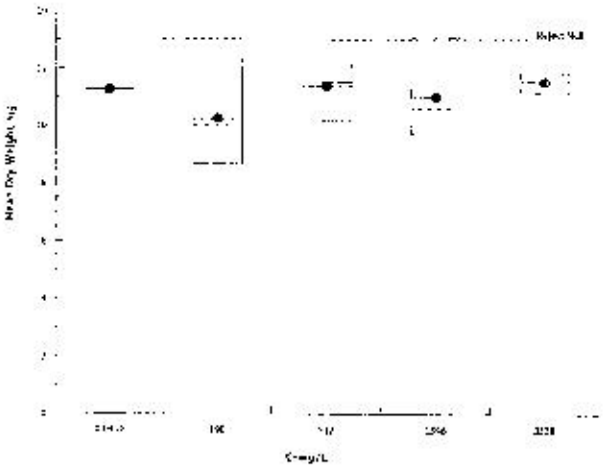
Lithobates pipiens 10-Day

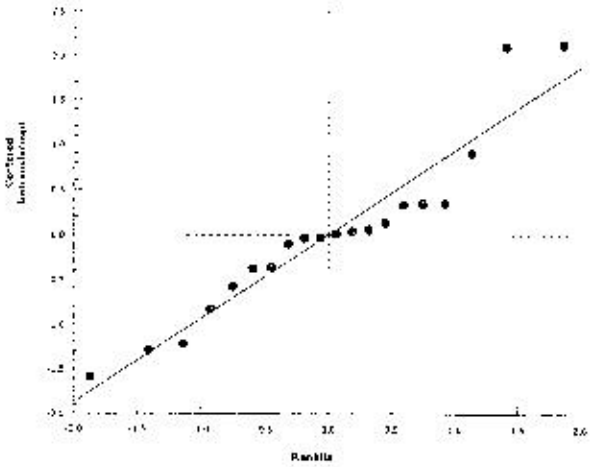
Report Date:	24 Feb-16 15:09 (p 2 of 2)		
Test Code:	468-010LF 17-7859-8882		

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length-mm TRE Environmental Strategies, LLC

Analysis ID:	10-2588-2337	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:09	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





DM 2/24/16 E

Right Tibio - Fibula

CETIS Analytical Report

Lithobates pipiens 7C-Day

Report Date: 24 Feb-16 15:09 (p 1 of 2)
Test Code: 468-010RtTF | 04-4687-2260

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length - mm

TRE Environmental Strategies, LLC

Analysis ID:	05-3643-3144	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:09	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 7D	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Moo-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~<24~~

Sample ID:	20-6980-6581	Code:	7B5EBA0D	Client:	Confidential
Sample Date:	~~27-Oct-15 11:37~~	Material:	~~Not Applicable~~ NTO	Project:	Special Studies
Receive Date:	~~27-Oct-15 11:37~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	15.0%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	1.652	2.36	1.877	6	0.1630	CDF	Non-Significant Effect
0.04		517	-0.2975	2.36	1.877	6	0.8818	CDF	Non-Significant Effect
0.04		1346	0.3721	2.36	1.877	6	0.6598	CDF	Non-Significant Effect
0.04		3338	0.3362	2.36	1.877	6	0.6752	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.631334	1.407833	4	1.109	0.3886	Non-Significant Effect
Error	19.04738	1.269825	15			
Total	24.67871		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.384	13.3	0.1169	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9781	0.866	0.9076	Normal Distribution

~~Mean Dry Weight-mg~~ Summary Length - mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	12.5	11.82	13.18	12.55	11.93	12.96	0.2127	3.4%	0.0%
190		4	11.18	8.807	13.56	11.6	9.175	12.35	0.7464	13.3%	10.5%
517		4	12.74	10.9	14.57	12.97	11.18	13.82	0.5774	9.07%	-1.9%
1346		4	12.2	9.71	14.69	11.73	10.89	14.45	0.7831	12.8%	2.37%
3338		4	12.23	11.61	12.86	12.19	11.8	12.75	0.1962	3.21%	2.14%

~~Mean Dry Weight-mg~~ Detail Length - mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	11.93	12.51	12.59	12.96
190		12.29	10.92	9.175	12.35
517		13.82	13.35	12.6	11.18
1346		10.89	12	11.47	14.45
3338		12.15	11.8	12.75	12.23

D BML 2/24/16 E

Right Tibio-Fibula

CETIS Analytical Report

Lithobates pipiens 10-Day

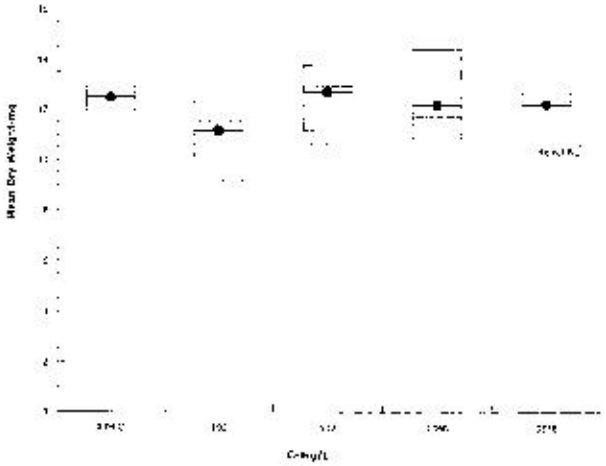
Report Date:	24 Feb-16 15:09 (p 2 of 2)
Test Code:	468-010RITF | 04-4687-2260

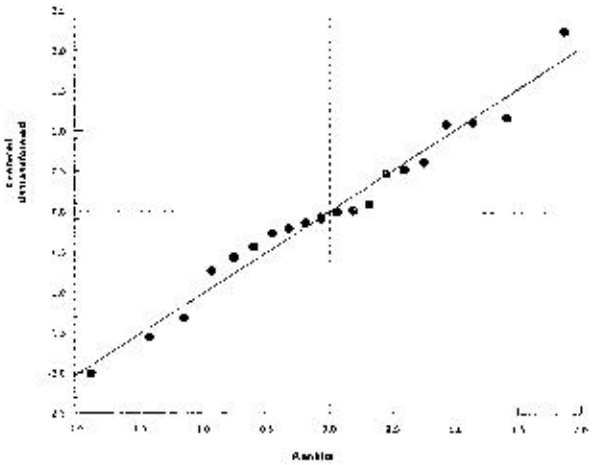
~~Fathead Minnow 7 d~~ Larval Survival and Growth Test Length-mm

TRE Environmental Strategies, LLC

Analysis ID:	05-3643-3144	**Endpoint:**	~~Mean Dry Weight-mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:09	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Demo 4 2016 E

Right Tibio - Fibula

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 15:10 (p 1 of 2)
Test Code: 468-010RtTF 04-4687-2250

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length-mm

TRE Environmental Strategies, LLC

Analysis ID:	14-0951-3013	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:10	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival ~~(7d)~~ 70d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	~~7-224~~

Sample ID:	20-6980-5581	Code:	7B5EBADD	Client:	Confidential
Sample Date:	~~27-Oct-15 11:37~~	Material:	~~Not Applicable~~ NTD	Project:	Special Studies
Receive Date:	~~27-Oct-15 11:37~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C < T	NA	NA	15.0%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-1.652	2.36	1.877	6	0.9956	CDF	Non-Significant Effect
0.04		517	0.2975	2.36	1.877	6	0.6906	CDF	Non-Significant Effect
0.04		1346	-0.3721	2.36	1.877	6	0.8977	CDF	Non-Significant Effect
0.04		3338	-0.3352	2.36	1.877	6	0.8901	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.631334	1.407833	4	1.109	0.3886	Non-Significant Effect
Error	19.04738	1.269825	15			
Total	24.67871		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.384	13.3	0.1169	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9781	0.866	0.9076	Normal Distribution

~~Mean Dry Weight-mg~~ Summary: Length-mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	12.5	11.82	13.18	12.55	11.93	12.96	0.2127	3.4%	0.0%
190		4	11.18	8.607	13.56	11.6	9.175	12.35	0.7464	13.3%	10.5%
517		4	12.74	10.9	14.57	12.97	11.18	13.62	0.5774	9.07%	-1.9%
1346		4	12.2	9.71	14.69	11.73	10.89	14.45	0.7831	12.8%	2.37%
3338		4	12.23	11.61	12.86	12.19	11.8	12.75	0.1962	3.21%	2.14%

~~Mean Dry Weight-mg~~ Detail: Length-mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	11.93	12.51	12.59	12.96
190		12.29	10.97	9.175	12.35
517		13.82	13.35	12.6	11.18
1346		10.89	12	13.47	14.45
3338		12.15	11.8	12.75	12.23

DM 2/24/16 E

Right Tibio-Fibula

CETIS Analytical Report

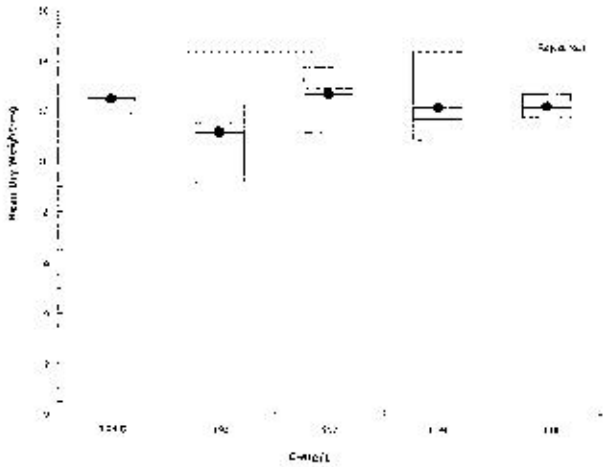
Lithobates pipiens 70-Day

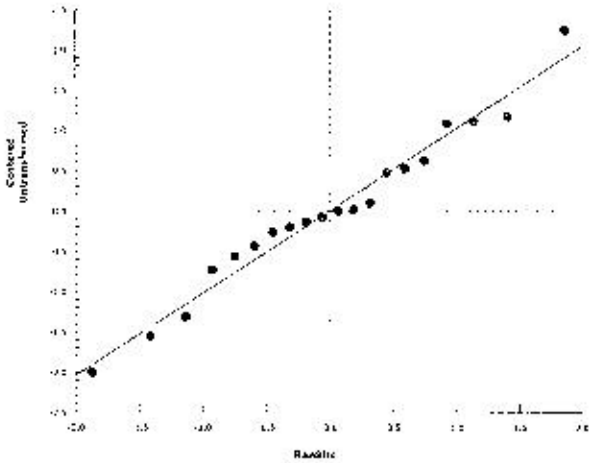
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Length - mm

Report Date:	24 Feb-16 15:10 (p 2 of 2)		
Test Code:	468-010RtTF | 04-4687-2260		
TRE Environmental Strategies, LLC			
Analysis ID:	14-0951-3013	**Endpoint:**	~~Mean Dry Weight-mg~~
Analyzed:	24 Feb-16 15:10	**Analysis:**	Parametric-Control vs Treatments
CETIS Version:	CETISv1.8.7		
Official Results:	Yes		

Graphics





DMM 2/24/16 E

Left Tibio - Fibula

CETIS Analytical Report

Lithobates pipiens 70-Day

Report Date: 24 Feb-16 15:10 (p 1 of 2)
Test Code: 468-010LTF 07-7951-3117

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length-mm

TRE Environmental Strategies, LLC

Analysis ID:	07-9201-3298	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:10	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	06-7170-1002	Test Type:	Growth-Survival (7d) 70 d	Analyst:	Lab Tech
Start Date:	20 May-15 12:00	Protocol:	Not Applicable	Diluent:	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	Species:	~~Not Applicable~~ Lithobates pipiens	Brine:	
Duration:	69d 19h	Source:	Carolina Biological Supply	Age:	<2~~8~~

Sample ID:	02-8515-2190	Code:	10FF13BE	Client:	Confidential
Sample Date:	~~27 Oct-15 11:50~~	Material:	~~Not Applicable~~ NTD	Project:	Special Studies
Receive Date:	~~27 Oct-15 11:50~~	Source:	Research		
Sample Age:	~~NA~~	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	16.2%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	1.659	2.36	2.028	6	0.1612	CDF	Non-Significant Effect
0.04		517	-0.4008	2.36	2.028	6	0.9035	CDF	Non-Significant Effect
0.04		1346	0.2844	2.36	2.028	6	0.6960	CDF	Non-Significant Effect
0.04		3338	0.06148	2.36	2.028	6	0.7795	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.357879	1.83947	4	1.242	0.3355	Non-Significant Effect
Error	22.22362	1.481575	15			
Total	29.5815		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.071	13.3	0.0890	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9574	0.866	0.4925	Normal Distribution

~~Mean Dry Weight-mg~~ Summary Length-mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	12.48	11.84	13.13	12.54	11.97	12.89	0.2032	3.26%	0.0%
190		4	11.06	7.701	14.41	11.24	8.375	13.37	1.054	19.1%	11.4%
517		4	12.83	11.46	14.19	13.02	11.7	13.58	0.429	6.69%	-2.76%
1346		4	12.24	10.17	14.31	11.8	11.23	14.13	0.6502	10.6%	1.96%
3338		4	12.43	11.46	13.4	12.39	11.73	13.22	0.3042	4.89%	0.42%

~~Mean Dry Weight-mg~~ Detail Length-mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	11.97	12.37	12.89	12.71
190		11.85	10.63	8.375	13.37
517		13.58	13.4	12.63	11.7
1346		11.23	12.04	11.57	14.13
3338		12.35	11.73	13.22	12.43

DM 2/24/16 E

Left Tibio-Fibula

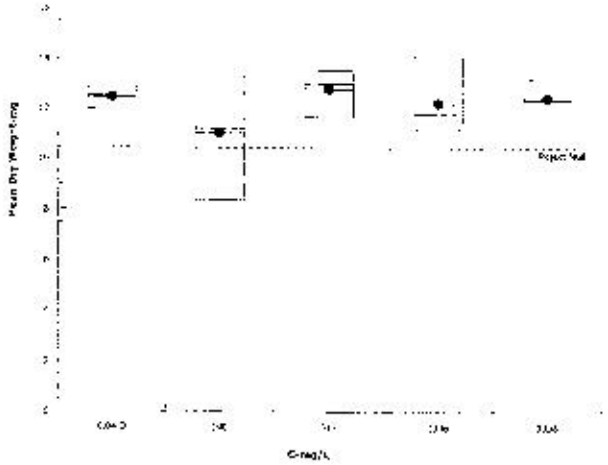
CETIS Analytical Report

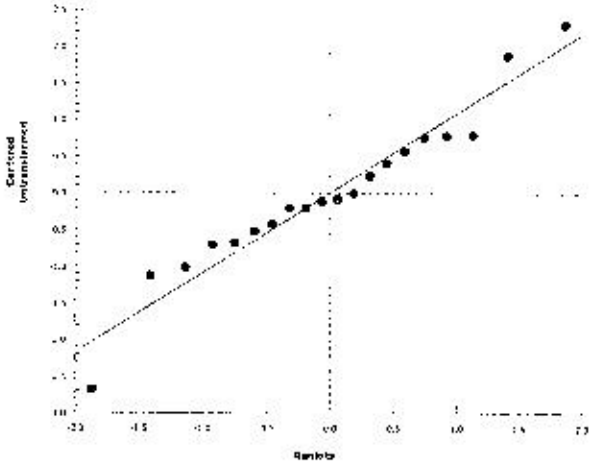
Report Date: 24 Feb-16 15:10 (p 2 of 2)
Test Code: 468-010LTF | 07-7961-3117

Lithobates pipiens 10-Day
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test
length-mm
TRE Environmental Strategies, LLC

Analysis ID:	07-9201-3298	**Endpoint:**	~~Mean Dry Weight-mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:10	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





DM 2/24/16 F

Left Tibio-Fibula

CETIS Analytical Report

Lithobates pipiens 10-Day

Report Date: 24 Feb-16 15:10 (p 1 of 2)
Test Code: 468-010LTF | 07-7961-3117

~~Fathead Minnow 7-d~~ Larval Survival and Growth Test Length-mm TRE Environmental Strategies, LLC

Analysis ID:	16-2122-0607	**Endpoint:**	~~Mean Dry Weight-mg~~	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Feb-16 15:10	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	05-7170-1002	**Test Type:**	Growth-Survival ~~(7d)~~ 10 d	**Analyst:**	Lab Tech
Start Date:	20 May-15 12:00	**Protocol:**	Not Applicable	**Diluent:**	Mod-Hard Synthetic Water
Ending Date:	29 Jul-15 07:00	**Species:**	~~Not Applicable~~ Lithobates pipiens	**Brine:**	
Duration:	69d 19h	**Source:**	Carolina Biological Supply	**Age:**	~~<24h~~

Sample ID:	02-8515-2190	**Code:**	10FF13BE	**Client:**	Confidential
Sample Date:	~~27 Oct-15 11:50~~	**Material:**	~~Not Applicable~~ NTO	**Project:**	Special Studies
Receive Date:	~~27 Oct-15 11:50~~	**Source:**	Research		
Sample Age:	~~NA~~	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	16.2%	3338	>3338	NA	

Dunnett Multiple Comparison Test

Control	vs	C-mg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
0.04		190	-1.659	2.36	2.028	6	0.9957	CDF	Non-Significant Effect
0.04		517	0.4008	2.36	2.028	6	0.6475	CDF	Non-Significant Effect
0.04		1346	-0.2844	2.36	2.028	6	0.8788	CDF	Non-Significant Effect
0.04		3338	-0.06148	2.36	2.028	6	0.8193	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.357879	1.83947	4	1.242	0.3355	Non-Significant Effect
Error	22.22362	1.481575	15			
Total	29.5815		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.071	13.3	0.0890	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9574	0.866	0.4925	Normal Distribution

~~Mean Dry Weight-mg~~ **Summary** : Length-mm

C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.04	Dilution Water	4	12.48	11.84	13.13	12.54	11.97	12.89	0.2032	3.26%	0.0%
190		4	11.06	7.701	14.41	11.24	8.375	13.37	1.054	19.1%	11.4%
517		4	12.83	11.46	14.19	13.02	11.7	13.58	0.429	6.69%	-2.76%
1346		4	12.24	10.17	14.31	11.8	11.23	14.13	0.6502	10.6%	1.96%
3338		4	12.43	11.46	13.4	12.39	11.73	13.22	0.3042	4.89%	0.42%

~~Mean Dry Weight-mg~~ **Detail** : Length-mm

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0.04	Dilution Water	11.97	12.37	12.89	12.71
190		11.85	10.63	8.375	13.37
517		13.58	13.4	12.63	11.7
1346		11.23	12.04	11.57	14.13
3338		12.35	11.73	13.22	12.43

DEME 3/24/16 E

Left Tibio-Fibula

CETIS Analytical Report

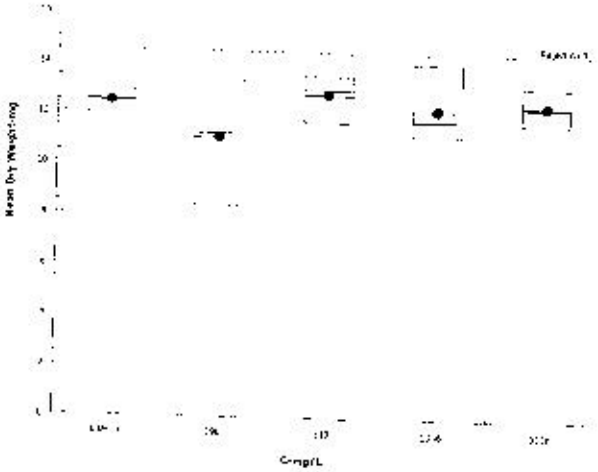
Lithobates pipiens 10-Day

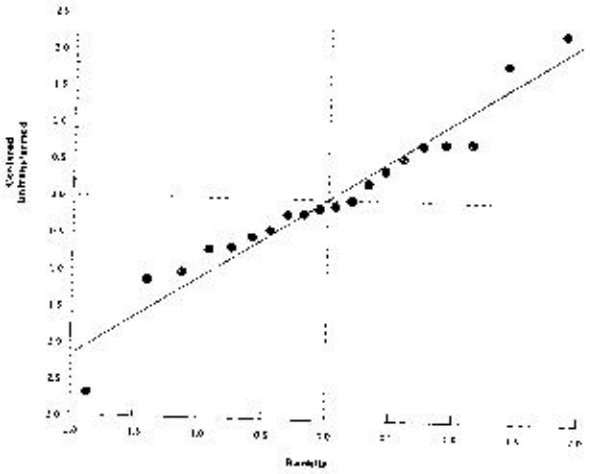
~~Fathead Minnow 7-d~~ Larval Survival and Growth Test

Length-mm

				Report Date:	24 Feb-16 15:10 (p 2 of 2)
				Test Code:	468-010LTF ; 07-7961-3117
					TRE Environmental Strategies, LLC
Analysis ID:	16-2122-0607	Endpoint:	~~Mean Dry Weight-mg~~	CETIS Version:	CETISv1.8.7
Analyzed:	24 Feb-16 15:10	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Graphics





Descriptive Statistics for Treatment = 0

	LimbErupt	Limbmin	Metamin	Metamor
N	4	4	4	4
Mean	46.300	43.750	45.250	50.950
SD	3.5467	0.9574	1.5000	3.9383
SE Mean	1.7744	0.4787	0.7500	1.9691
C.V.	7.6646	2.3495	3.3149	7.7297
Minimum	42.500	43.000	44.000	47.500
Median	45.850	43.500	45.000	49.900
Maximum	51.000	42.000	47.000	56.500

Descriptive Statistics for Treatment = 190

	LimbErupt	Limbmin	Metamin	Metamor
N	4	4	4	4
Mean	53.250	44.750	49.250	55.793
SD	1.5679	2.6300	3.5940	1.2710
SE Mean	0.7840	1.3150	1.7970	0.6355
C.V.	2.9444	5.8770	7.2974	2.2782
Minimum	52.000	41.000	44.000	54.670
Median	52.975	45.500	50.500	55.500
Maximum	55.250	47.000	52.000	57.500

Descriptive Statistics for Treatment = 517

	LimbErupt	Limbmin	Metamin	Metamor
N	4	4	4	4
Mean	50.375	43.000	47.750	53.540
SD	3.3696	2.5820	3.5000	1.3689
SE Mean	1.6848	1.2910	1.7500	0.6844
C.V.	6.6890	6.0046	7.3298	2.5567
Minimum	46.250	40.000	44.000	51.500
Median	51.000	43.000	47.500	54.165
Maximum	53.250	46.000	52.000	54.330

Descriptive Statistics for Treatment = 1346

	LimbErupt	Limbmin	Metamin	Metamor
N	4	4	4	4
Mean	49.738	43.500	48.000	54.563
SD	3.9046	3.1097	3.9158	4.3893
SE Mean	1.9523	1.5546	1.9579	2.1946
C.V.	7.8503	7.1474	8.1579	8.0445
Minimum	45.500	41.000	44.000	51.000
Median	49.525	42.500	47.500	53.635
Maximum	54.400	48.000	53.000	60.300

Descriptive Statistics for Treatment = 3338

	LimbErupt	Limbmin	Metamin	Metamor
N	4	4	4	4
Mean	59.165	52.000	58.000	61.918
SD	4.5727	5.4772	5.0990	5.0320
SE Mean	2.2864	2.7386	2.5495	2.5160
C.V.	7.7287	10.533	8.7914	8.1269
Minimum	54.000	45.000	51.000	57.000
Median	59.165	52.500	59.000	61.000
Maximum	64.330	58.000	63.000	68.670

DOP 3/4/16
QA: AR 3/4/16

Statistix 9.0 Days_StatistixData, 3/4/2016, 1:29:38 PM

Shapiro-Wilk Normality Test

Variable	N	W	P
Metamin	20	0.8784	0.0266
LimbErupt	20	0.9638	0.6223
Limbmin	20	0.8557	0.0066 ← fails Normality Test
Metamor	20	0.9451	0.2982

DAP 3/4/16
GRA. AHS 3/4/16

Statistix 9.0 Days_StatistixData, 10/26/2015, 6:15:11 PM

One-Way AOV for LimbErupt by Treatment

Source	DF	SS	MS	F	P
Treatment	4	371.497	92.8743	7.42	0.0017
Error	15	187.683	12.5122		
Total	19	559.180			

Signif. Diff.

Grand Mean 51.766 CV 6.83

Homogeneity of Variances	F	P
Levene's Test	1.23	0.3419
O'Brien's Test	0.78	0.5529
Brown and Forsythe Test	1.26	0.3282

Welch's Test for Mean Differences

Source	DF	F	P
Treatment	4.0	4.85	0.0337
Error	7.1		

Component of variance for between groups 20.0905
Effective cell size 4.0

Treatment	Mean
0	46.300
190	53.250
517	50.575
1346	49.738
3338	50.165

Observations per Mean 4
Standard Error of a Mean 1.7686
Std Error (Diff of 2 Means) 2.5012

DAP 10/27/15
CA: 12/9/15

One-sided Dunnett's Multiple Comparisons with a Control OF LimbErupt
Control: Treatment=0

Simultaneous 95% confidence intervals of treatment mean - control mean

Treatment	Mean	Lower Bound	Difference
0	46.300		
190	53.250	1.057	6.950*
517	50.375	-1.818	4.075
1346	49.738	-2.450	3.437
3338	59.165	6.977	12.865*

Alpha	0.05	Standard Error for Comparison	2.5012
Critical D Value	2.356	Critical Value for Comparison	5.8934

3/4/16 3/4/16

Statistix 9.0 Days_StatistixData, 10/27/2015, 7:53:50 AM

One-Way AOV for Metamor by Treatment

Source	DF	SS	MS	F	P
Treatment	4	266.336	66.5841	5.24	0.0076
Error	15	190.757	12.7171		
Total	19	457.093			

Signif. Diff

Grand Mean 55.552 CV 6.44

Homogeneity of Variances	F	P
Levene's Test	1.72	0.1969
O'Brien's Test	1.10	0.3908
Brown and Forsythe Test	1.80	0.1808

Welch's Test for Mean Differences

Source	DF	F	P
Treatment	4.0	3.44	0.0731
Error	7.1		

Component of variance for between groups 13.4667
Effective cell size 4.0

Treatment	Mean
0	50.950
190	55.799
517	53.540
1346	54.563
3338	61.925

Observations per Mean 4
Standard Error of a Mean 1.7831
Std Error (Diff of 2 Means) 2.5216

DVC 10/27/15
GM 10/29/15

One-sided Dunnett's Multiple Comparisons with a Control OF Metamor
Control: Treatment=0

Simultaneous 95% confidence intervals of treatment mean - control mean

Treatment	Mean	Lower Bound	Difference
0	50.950		
190	55.793	-1.099	4.843
817	53.540	-3.351	2.590
1346	54.563	-2.329	3.612
3333	61.918	5.026	10.968*

Alpha	0.05	Standard Error for Comparison	2.5216
Critical T Value	2.356	Critical Value for Comparison	5.9415

DAP 3/4/16
GK 3/4/16

Statistix 9.0 Days_StatistixData, 10/27/2015, 7:56:35 AM

Kruskal-Wallis One-Way Nonparametric AOV for LimbMin by Treatment

Treatment	Mean Rank	Sample Size
0	4.5	4
190	12.0	4
517	8.9	4
1346	10.0	4
2338	17.4	4
Total	10.5	20

Kruskal-Wallis Statistic 10.5648
P-Value, Using Chi-Squared Approximation 0.0319

Parametric AOV Applied to Ranks

Source	DF	SS	MS	F	P
Between	4	365.875	91.4688	4.70	0.0117
Within	15	292.125	19.4750		
Total	19	658.000			

Significant

Total number of values that were tied 13
Max. diff. allowed between ties 0.00001

Cases Included 20 Missing Cases 0

DEA 10/27/15
MA 12/31/15

Statistix 9.0 Day6_StatistixData. 10/27/2015, 7:56:51 AM

Kruskal-Wallis All-Pairwise Comparisons Test of LimbMin by Treatment

Treatment	Rank Mean	Homogeneous Groups
3338	17.375	A
190	12.000	AB
1346	10.000	AB
517	8.5750	AB
0	4.3500	B

Alpha 0.05
Critical Z Value 2.807 Critical Value for Comparison 11.743
There are 2 groups (A and B) in which the means
are not significantly different from one another.

DAP 10/27/15
BN_AE 12/9/15

Statistix 9.0 Days StatistixData, 3/4/2016, 1:34:27 PM

One-Way AOV for Metamin by Treatment

Source	DF	SS	MS	F	P	
Treatment	4	382.300	95.5750	6.95	0.0023	← SIG. DIFF
Error	15	206.250	13.7500			
Total	19	588.550				

Grand Mean 49.650 CV 7.47

Homogeneity of Variances	F	P
Levene's Test	0.95	0.4601
O'Brien's Test	0.61	0.6010
Brown and Forsythe Test	0.88	0.6848

Welch's Test for Mean Differences

Source	DF	F	P
Treatment	4.0	5.07	0.0309
Error	7.0		

Component of variance for between groups 20.4563
Effective cell size 4.0

Treatment	Mean
0	43.250
190	49.250
517	47.750
1346	49.000
3338	58.000

Observations per Mean 4
Standard Error of a Mean 1.8540
Std Error (Diff of 2 Means) 2.6220

DAP 3/4/16
QM: AR 3/4/16

One-sided Dunnett's Multiple Comparisons with a Control OF Metamin
Control: Treatment=0

Simultaneous 95% confidence intervals of treatment mean - control mean

Treatment	Mean	Lower Bound	Difference
0	45.250		
190	49.250	-2.178	4.000
417	47.750	-3.678	2.500
1346	48.000	-3.428	2.750
2538	58.000	6.572	12.750*

Alpha	0.05	Standard Error for Comparison	2.6220
Critical D Value	2.356	Critical Value for Comparison	6.1781

QAP 3/4/16
QA: AC 3/4/16

Descriptive Statistics for Conc = 0

	Pheart	Pkidney	Pliver	Plung	Pspleen
N	3	3	3	3	3
Mean	6.120E-03	9.640E-03	0.0397	8.410E-03	3.900E-04
SD	5.565E-04	6.236E-04	1.715E-03	2.285E-03	7.937E-05
Variance	3.097E-07	3.889E-07	2.940E-06	5.235E-06	6.300E-09
SE Mean	3.213E-04	3.600E-04	9.902E-04	1.321E-03	4.583E-05
C.V.	9.0933	6.4691	4.3231	27.206	20.342
Minimum	5.640E-03	9.270E-03	0.0380	5.910E-03	3.300E-04
Median	5.990E-03	9.290E-03	0.0397	8.920E-03	3.600E-04
Maximum	6.730E-03	0.0104	0.0414	0.0104	4.800E-04

Values of proportional values divided against spleen values. DAP 3/2/16

	Theart	Tkidney	Tliver	Tlung	Tspleen
N	3	3	3	3	3
Mean	0.0783	0.0983	0.2005	0.0913	0.0197
SD	3.545E-03	3.163E-03	4.394E-03	0.0129	1.970E-03
Variance	1.256E-05	1.000E-05	1.930E-05	1.673E-04	3.881E-06
SE Mean	2.047E-03	1.826E-03	2.537E-03	7.467E-03	1.137E-03
C.V.	4.5297	3.2178	2.1914	14.176	10.009
Minimum	0.0732	0.0964	0.1961	0.0770	0.0182
Median	0.0775	0.0965	0.2006	0.0946	0.0190
Maximum	0.0821	0.1020	0.2049	0.1022	0.0219

All "T-" variables are the arcsine ($\sqrt{x}$) transformation of proportional organ wt. values

Descriptive Statistics for Conc = 190

	Pheart	Pkidney	Pliver	Plung	Pspleen
N	3	3	3	3	3
Mean	6.210E-03	0.0113	0.0408	9.673E-03	4.600E-04
SD	3.799E-04	7.217E-04	4.413E-03	1.660E-03	9.849E-05
Variance	1.443E-07	5.209E-07	1.948E-05	2.754E-06	9.700E-09
SE Mean	2.193E-04	4.167E-04	2.548E-03	9.582E-04	5.686E-05
C.V.	6.1170	6.3814	10.820	17.157	21.411
Minimum	5.800E-03	0.0108	0.0358	8.090E-03	3.800E-04
Median	6.280E-03	0.0110	0.0422	9.830E-03	4.300E-04
Maximum	6.550E-03	0.0121	0.0443	0.0114	5.700E-04

	Theart	Tkidney	Tliver	Tlung	Tspleen
N	3	3	3	3	3
Mean	0.0789	0.1065	0.2032	0.0963	0.0214
SD	2.429E-03	3.394E-03	0.0132	8.465E-03	2.258E-03
Variance	5.899E-06	1.152E-05	1.276E-04	7.166E-05	5.100E-06
SE Mean	1.402E-03	1.959E-03	6.823E-03	4.887E-03	1.303E-03
C.V.	3.0798	3.1773	6.5619	8.6139	10.568
Minimum	0.0762	0.1043	0.1905	0.0901	0.0195
Median	0.0793	0.1049	0.2069	0.0978	0.0207
Maximum	0.0810	0.1104	0.2121	0.1070	0.0239

Descriptive Statistics for Conc = 517

	Pheart	Pkidney	Pliver	Plung	Pspleen
N	3	3	3	3	3
Mean	5.983E-03	8.307E-03	0.0424	4.537E-03	4.233E-04
SD	3.014E-04	4.500E-04	3.338E-03	4.368E-04	7.024E-05
Variance	9.082E-08	2.070E-07	1.114E-05	1.908E-07	4.933E-09
SE Mean	1.740E-04	2.627E-04	1.927E-03	2.522E-04	4.055E-05
C.V.	5.0371	5.4149	7.8773	9.6292	16.592
Minimum	5.700E-03	7.900E-03	0.0394	4.170E-03	3.500E-04
Median	5.950E-03	8.360E-03	0.0417	4.420E-03	4.300E-04
Maximum	6.300E-03	8.810E-03	0.0460	5.020E-03	4.900E-04

	Theart	Tkidney	Tliver	Tlung	Tspleen
N	3	3	3	3	3
Mean	0.0774	0.0915	0.2072	0.0674	0.0205
SD	1.951E-03	2.501E-03	8.048E-03	3.225E-03	1.724E-03

DAP 3/2/16
CL. AR 3/3/16

Variance	3.605E-06	6.253E-06	6.799E-05	1.040E-05	2.971E-06
SE Mean	7.120E-03	1.443E-02	4.761E-03	1.862E-03	9.952E-04
C.V.	2.5198	2.7322	3.9791	4.7882	8.3970
Minimum	0.0756	0.0890	0.1999	0.0646	0.0137
Median	0.0772	0.0916	0.2056	0.0665	0.0237
Maximum	0.0795	0.0940	0.2162	0.0709	0.0221

Descriptive Statistics for Conc = 1346

	Pheart	Pkidney	Pliver	Plung	Pspleen
N	3	3	3	3	3
Mean	6.573E-03	9.840E-03	0.0437	7.420E-03	4.200E-04
SD	2.219E-04	3.822E-04	3.445E-03	7.916E-04	1.216E-04
Variance	4.923E-08	1.461E-07	1.186E-05	6.267E-07	1.480E-08
SE Mean	1.281E-04	2.207E-04	1.989E-03	4.571E-04	7.024E-05
C.V.	3.3765	3.8845	7.8764	10.669	28.966
Minimum	6.370E-03	9.590E-03	0.0400	6.890E-03	3.400E-04
Median	6.540E-03	9.620E-03	0.0443	7.040E-03	3.600E-04
Maximum	6.810E-03	0.0102	0.0468	8.330E-03	5.600E-04

	Theart	Tkidney	Tliver	Tlung	Tspleen
N	3	3	3	3	3
Mean	0.0812	0.0992	0.2100	0.0862	0.0204
SD	1.370E-03	1.926E-03	8.469E-03	4.551E-03	2.876E-03
Variance	1.878E-06	3.710E-06	7.173E-05	2.071E-05	8.269E-06
SE Mean	7.913E-04	1.112E-03	4.890E-03	2.627E-03	1.660E-03
C.V.	1.6868	1.9387	4.0319	5.2814	14.123
Minimum	0.0799	0.0981	0.2014	0.0831	0.0184
Median	0.0813	0.0984	0.2122	0.0840	0.0190
Maximum	0.0826	0.1016	0.2182	0.0914	0.0237

Descriptive Statistics for Conc = 3338

	Pheart	Pkidney	Pliver	Plung	Pspleen
N	3	3	3	3	3
Mean	6.730E-03	0.0130	0.0372	0.0117	6.000E-04
SD	3.716E-04	2.214E-03	4.271E-03	2.005E-03	7.937E-05
Variance	1.383E-07	4.900E-06	1.824E-05	4.021E-06	6.300E-09
SE Mean	2.146E-04	1.278E-03	2.466E-03	1.157E-03	4.583E-05
C.V.	5.5200	16.980	11.491	17.144	13.229
Minimum	6.370E-03	0.0109	0.0338	9.710E-03	5.400E-04
Median	6.680E-03	0.0129	0.0387	0.0117	5.700E-04
Maximum	7.110E-03	0.0152	0.0420	0.0137	6.900E-04

	Theart	Tkidney	Tliver	Tlung	Tspleen
N	3	3	3	3	3
Mean	0.0820	0.1142	0.1938	0.1081	0.0245
SD	3.270E-03	9.755E-03	0.0112	9.353E-03	1.598E-03
Variance	3.134E-06	9.515E-05	1.246E-04	8.745E-05	2.554E-06
SE Mean	1.310E-03	5.632E-03	6.445E-03	5.399E-03	9.226E-04
C.V.	3.7670	8.5451	5.7600	8.6510	6.5327
Minimum	0.0799	0.1046	0.1850	0.0987	0.0232
Median	0.0818	0.1138	0.1900	0.1082	0.0239
Maximum	0.0844	0.1247	0.2064	0.1174	0.0263

DAP 3/2/16
QA. AR 3/3/16.

Shapiro-Wilk Normality Test

Variable	N	W	P
Heart	15	0.9646	0.7709
Kidney	15	0.9413	0.2995
Liver	15	0.9682	0.8306
Lung	15	0.9709	0.8717
Testes	15	0.9237	0.2116

All parametric analyses

DAP 3/2/16
GA. AR 3/3/16

Statistix 9.0 Organ wts_Transformed, 2/26/2016, 12:57:11 PM

One-Way AOV for Theart by Conc

Source	DF	SS	MS	F	P
Conc	4	4.659E-05	1.164E-05	1.99	0.1775
Error	10	5.860E-05	5.860E-06		
Total	14	1.052E-04			

← No Diff.

Grand Mean 0.0795 CV 3.04

Homogeneity of Variances	F	P
Levene's Test	1.28	0.3082
O'Brien's Test	0.61	0.6624
Brown and Forsythe Test	0.34	0.8486

Pass homogeneity of variance test

Welch's Test for Mean Differences

Source	DF	F	P
Conc	4.0	1.99	0.2366
Error	4.9		

Component of variance for between groups 1.929E-06
Effective cell size 3.0

Conc	Mean
0	0.0793
190	0.0789
517	0.0774
1346	0.0812
3538	0.0820

Observations per Mean 3
Standard Error of a Mean 1.397E-03
Std Error (Diff of 2 Means) 1.977E-03

DAP 3/2/16
QA. AR 3/3/16

Statistix 9.0 Organ wts_Transformed, 2/26/2016, 1:25:13 PM

One-Way AOV for Tlung by Conc

Source	DF	SS	MS	F	P
Conc	4	0.00277	6.936E-04	9.70	0.0013
Error	10	0.00071	7.150E-05		
Total	14	0.00349			

← Diff (see attached multi-comparison test)

Grand Mean 0.0902 CV 9.37

Homogeneity of Variances	F	P
Levene's Test	1.91	0.1861
O'Brien's Test	0.85	0.5267
Brown and Forsythe Test	0.66	0.6334

Pass variance test

Welch's Test for Mean Differences

Source	DF	F	P
Conc	4.0	16.45	0.0054
Error	4.7		

Component of variance for between groups 2.074E-04
Effective cell size 3.0

Conc	Mean
0	0.0917
190	0.0983
517	0.0674
1346	0.0862
3338	0.1081

← Signif Diff from control

Observations per Mean 3
Standard Error of a Mean 4.882E-03
Std Error (Diff of 2 Means) 6.904E-03

DAP 3/2/16
QA: MB 3/3/16

Statistix 9.0 Organ was Transformed, 2/26/2016, 1:28:57 PM

Tukey HSD All-Pairwise Comparisons Test of Tlung by Conc

Conc	Mean	Homogeneous Groups
3338	0.1081	A
193	0.0983	A
0	0.0912	A
1346	0.0862	AB
517	0.0674	B ← This conc. is signif. diff. from the control

Alpha 0.05 Standard Error for Comparison 6.9348 03
Critical Q Value 4.655 Critical Value for Comparison 0.0227
There are 2 groups (A and B) in which the means
are not significantly different from one another.

DAP 3/2/16
QA AR 3/2/16

Statistix 9.0 Organ wts_Transformed, 2/26/2016, 1:50:06 PM

One-Way AOV for Tspleen by Conc

Source	DF	SS	MS	F	P
Conc	4	4.928E-05	1.057E-05	2.32	0.1278
Error	10	4.555E-05	4.555E-06		
Total	14	9.783E-05			

← Not signif. Different

Grand Mean 0.0213 CV 10.03

Homogeneity of Variances	F	P
Levene's Test	0.84	0.5285
O'Brien's Test	0.37	0.8275
Brown and Forsythe Test	0.09	0.9840

Pass variance test

Welch's Test for Mean Differences

Source	DF	F	P
Conc	4.0	2.53	0.1691
Error	5.0		

Component of variance for between groups 2.008E-06
Effective cell size 3.0

Conc	Mean
0	0.0197
190	0.0214
517	0.0205
1346	0.0204
3338	0.0245

Observations per Mean 3
Standard Error of a Mean 1.232E-03
Std Error (Diff of 2 Means) 1.743E-03

DAP 3/2/16
QA. AR 3/3/16

Tukey HSD All-Pairwise Comparisons Test of Tkidney by Conc

Conc	Mean	Homogeneous Groups
2328	0.1148	A
190	0.1065	AB
1346	0.0993	DC
0	0.0983	BC
517	0.0915	C

High concn. is signif. greater than Control, 517, + 1346 mg/L

Alpha 0.05 Standard Error for Comparison 4.108E-03
Critical Q Value 4.655 Critical Value for Comparison 0.0135
There are 3 groups (A, B, etc.) in which the means are not significantly different from one another.

DAO 3/3/16
SA: AR 3/3/16

Statistix 9.0 Organ wts_Transformed, 2/29/2016, 4:31:06 PM

One-sided Dunnett's Multiple Comparisons with a Control OF Tkidney
Control: Conc=0

Simultaneous 95% confidence intervals of treatment mean - control mean

Conc	Mean	Lower Bound	Difference
0	0.0983		
190	0.1065	-0.0019	0.0082
517	0.0975	0.0169	-0.0068
1346	0.0993	-0.0091	0.0010
3328	0.1142	0.0057	0.0158*

In a 1-tail test, high concn. is signif. greater than the control

Alpha	0.05	Standard Error for Comparison	4.103E-03
Critical D Value	2.466	Critical Value for Comparison	0.0101

DAP 3/2/16
S.P. AR 3/3/16

Statistix 9.0 Organ wts_Transformed, 2/29/2016, 4:28:53 PM

One-Way AOV for Tkidney by Conc

Source	DF	SS	MS	F	P
Conc	4	0.00090	2.240E-04	8.85	0.0025
Error	10	0.00025	2.532E-05		
Total	14	0.00115			

← Signif. Diff. (See attached multi-comparison test)

Grand Mean 0.1020 CV 4.93

Homogeneity of Variances	F	P
Levene's Test	3.28	0.0579
O'Brien's Test	1.46	0.2852
Brown and Forsythe Test	1.28	0.3425

Pass variance test

Welch's Test for Mean Differences

Source	DF	F	P
Conc	4.0	8.59	0.0196
Error	4.9		

Component of variance for between groups 6.622E-05
Effective cell size 3.0

Conc	Mean
0	0.0983
190	0.1065
517	0.0918
1546	0.0993
2538	0.1142

Observations per Mean 3
Standard Error of a Mean 2.903E-03
Std Error (Diff of 2 Means) 4.108E-03

DAP 3/2/16
QA: AZ 3/7/16

Statistix 9.0 Organ wts Transformed, 2/29/2016, 5:23:06 PM

One-Way AOV for Tliver by Conc

Source	DF	SS	MS	F	P
Conc	4	0.00050	1.249E-04	1.52	0.2694
Error	10	0.00082	8.225E-05		
Total	14	0.00132			

← Not Sign. Diff.

Grand Mean 0.2030 CV 4.47

Homogeneity of Variances	F	P
Levene's Test	0.97	0.4660
O'Brien's Test	0.43	0.7837
Brown and Forsythe Test	0.22	0.9207

Pass variance test

Welch's Test for Mean Differences

Source	DF	F	P
Conc	4.0	1.09	0.4551
Error	4.8		

Component of variance for between groups 1.420E-05
Effective cell size 2.0

Conc	Mean
0	0.2005
190	0.2032
517	0.2072
1346	0.2106
3338	0.1958

Observations per Mean 3
Standard Error of a Mean 5.236E-03
Std Error (Diff of 2 Means) 7.405E-03

DAP 3/2/16
QA DAP 5/3/16 AR 5/3/16

US Army Public Health Center
Aberdeen Proving Ground, MD

Study Contract Title: One Generation Amphibian Study
W91ZLK-14-P-0372

APPENDIX E

Pathology Report
Army Public Health Center
Division of Toxicologic Pathology
Toxicological Study No. S.0040555-5

Prepared by

TRE Environmental Strategies

TRE Environmental Strategies, LLC
Fort Collins, CO

March 2016

TRE Project Number: 15001-468-710

DEPARTMENT OF THE ARMY
ARMY PUBLIC HEALTH CENTER (PROVISIONAL)
5158 BLACKHAWK ROAD
ABERDEEN PROVING GROUND MARYLAND 21010-5403

March 30, 2016

SUBJECT: Pathology Report for TRE Project No.: 15001-468, Protocol No. RP4OK.468.001, One-Generation Study of the Effects of 3-nitro-1,2,4-triazol-5-one (NTO) to the Northern Leopard Frog (*Lithobates pipiens*) Under Static Renewal Test Conditions, December2015

Dr. David Pillard
TRE Environmental Strategies Toxicology Laboratory
100 Racquette Drive
Fort Collins, Colorado 80524

Dear Dr. Pillard:

An electronic copy of the subject report has been provided.

Please contact us if this report or any of our services did not meet your expectations.

The Army Public Health Center (Provisional) point of contact is LTC Erica E. Carroll, Toxicology Directorate, Division of Toxicologic Pathology. She may be contacted at DSN 584-3980, commercial 410-436-3980 or by electronic mail at usarmy.apg.medcom-aphc.mbx.tox-info@mail.mil.

Sincerely,

Mark S. Johnson
Director, Toxicology

Enclosure

ARMY PUBLIC HEALTH CENTER (Provisional)

5158 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

Toxicological Study No. S.0040555-5, March 2016
Toxicology Portfolio
Division of Toxicologic Pathology

Pathology Report for One-Generation Study of the Effects of 3-nitro-1,2,4-triazol-5-one (NTO) to the Northern Leopard Frog (*Lithobates pipiens*) Under Static Renewal Test Conditions, December 2015

Protocol No. RP4OK.468.001
TRE Project No.: 15001-468

Prepared by:
Erica E. Carroll, DVM, PhD, Diplomate ACVP

Approved for public release; distribution unlimited.

Specialty: 500C, Toxicity Tests

Acknowledgement

Dr. Jeffrey C. Wolf, DVM, Diplomate ACVP, very kindly provided expertise and mentorship on anuran histopathology, for which this pathologist is very grateful.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This pathology investigation was conducted in a manner consistent with the principles of the United States Environmental Protection Agency (USEPA) Good Laboratory Practice regulations of the Toxic Substances Control Act (TSCA), as detailed in 40 CFR Part 792, plus amendments.

CARROLL.ERICA.E.1027432413

28 December 2015
Date

Erica E. Carroll, DVM, PhD, Diplomate ACVP
LTC, VC
Study Pathologist
Toxicology Portfolio
Army Public Health Center (Provisional)

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Toxicological Study No. S. 0040555-5
Protocol No. RP4OK.468.001
Pathology Report for
One-Generation Study of the Effects of 3-nitro-1,2,4-triazol-5-one (NTO) to the Northern Leopard Frog (*Lithobates pipiens*) Under Static Renewal Test Conditions
December 2015

1 Summary

1.1 Purpose

Larval (tadpole) anuran *Lithobates pipiens (*northern leopard frog, formerly recognized as *Rana pipiens)* were exposed to 3-nitro-1,2,4-triazol-5-one (NTO) or to NTO-free dilution water control. Organisms were exposed in water until the majority of control organisms emerged from the water as air-breathing froglets (up to 90 days), which is equivalent to Gosner Stage 46 (Gosner, 1960). All subjects were sacrificed on the day they reached Gosner Stage 46; that varied from ~45 days post-hatch (PH) in the controls to 60+ days PH in the high NTO concentration. Froglets were humanely euthanized using MS-222 and liver and kidney tissues dissected, fixed in formalin and shipped overnight to the Division of Toxicologic Pathology, Army Public Health Center (Provisional) (APHC (Prov)) for histologic processing and evaluation.

1.2 Authority

MIPR No. W74RDV53092356. This study was conducted with funding from the Strategic Environmental Research and Development Program (SERDP) (Project number ER-2223). This toxicology study addresses, in part, the environmental safety and occupational health requirements outlined in Army Regulations (AR) 200-1, AR 40-5, and AR 70-1; Department of Defense Instruction 4715.4; and Army Environmental Requirements and Technology Assessments (AERTA) [1-6].

2 References

Goff G.L., F.L. Frye, and E.R. Jacobson, eds. 1984. *Disease of Amphibians and Reptiles*. New York: Plenum Press. p. 627

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*. 16(3):183-190.

Norris D.A. and J.A. Carr, eds. 2013. *Vertebrate Endocrinology*, *5th Edition*. London, UK: Elsevier. p. 302.

3 Methods

Due to their small size, samples were placed whole into labelled cassettes with protective sponges, processed per standard operating procedures, embedded in paraffin, sectioned to 4 micrometers (um) thickness onto microscope slides, stained with hematoxylin and eosin, and cover-slipped.

4 Individual Animal Descriptions

- 00A1 Kidney (Control): Disrupting approximately 20% of the renal parenchyma (tubules and hematopoietic tissue) are multiple aggregates of flattened macrophages encircling necrotic cellular debris and granulocytes (granulomas). There is moderate amount of black pigment at the periphery and multifocally scattered within the section. About 10% of the section is autolyzed. There is ureter tissue with a few mitotic figures.

- 00A1 Kidney (Control): Granulomas, multifocal, mild. Liver (Control): Approximately 25% of the tissue is autolyzed. There is extracellular and intracellular pigment, unassociated with erythrocytes; therefore is not “formalin pigment” (acid hematin).

- 00B1 Kidney (Control): Abundant subcapsular hematopoiesis. Ovary with germ cells (primary oocytes) is present. There is a small amount of peripheral autolysis. Pigment is moderate, superficial, around the ovary and vessels. Ureteral tissue is present. Few small granulomas are present.

- 00B1 Kidney (Control): Granulomas, multifocal, minimal.
  Liver (Control): About 5% autolyzed. No observed lesions.

- 00B2 Kidney (Control): Pigment only at periphery, at capsule. Ureter is present.
  Liver (Control): 10% autolysis. No lesions observed.

- 00C1 Kidney (Control): Granulomas, multifocal, minimal.
  Liver (Control): No observed lesions.

- 00C2 Kidney (Control): Granulomas, multifocal, minimal.
  Liver (Control): No lesions observed.

- 20A1 Kidney (High): Granulomas, multiple, mild. Ovary with primary oocytes.
  Liver (High): No lesions observed.

- 20B1 Kidney (High): Granulomas, multiple, mild.
  Liver (High): No lesions observed.

- 20B2 Kidney (High): Granulomas, multifocal, moderate.
  Liver (High): Approximately 30% autolyzed. No lesions observed.

- 20C1 Kidney (High): Granulomas, multiple, mild.
  Liver (High): No lesions observed.

- 20C2 Kidney (High): Granulomas, multiple, mild. Ovarian tissue with oocytes.
  Liver (High): 10% autolyzed. No lesions observed.

5 Results

Histopathologic changes are itemized in Table 1. Pathology was absent in liver sections. Intranuclear vacuoles were present in liver sections in two control frogs, which are believed to be artifact. The only recognized pathology was in renal tissue, was present in five of five examined high-exposure frogs and four of five control frog tissues.

Table 1. Histopathologic Findings in NTO-Exposed Anurans

identity #'s-->	00A1	00B1	00B2	00C1	00C2	20A1	20B1	20B2	20C1	20C2
Treatment-->	CTRL	CTRL	CTRL	CTRL	CTRL	HIGH	HIGH	HIGH	HIGH	HIGH
Histopathologic Findings:										
KIDNEY										
Granulomas	2	1	0	1	1	2	2	3	2	2
LIVER										
Intranuclear vacuoles (seen at 10X)*	0	1	0	0	2	0	0	0	0	0

* 20C1-A few nuclei are refractile, like precursors to the change seen in 00C2. Suspect artifact.

Scoring Criteria: 0 means present in less than 1% of affected cell type. '1' (minimal) up to 5%; '2' (mild) 6-20%. '3' (moderate) 21-40% of cell type affected. '4' (marked) >40% of cell type affected.

6 Discussion

Granulomas were identified in all five high-exposure frogs and in four of five control frog kidneys. They were characterized by flattened macrophages or giant cells at the periphery of necrotic cellular debris, a few granulocytes with eosinophilic cytoplasmic granules. Granulomas or granulomatous inflammation is generally associated with acid-fast bacteria (such as mycobacteria) or fungal pathogens. Ziehl-Neelsen (an acid-fast stain) is used to detect mycobacteria; Gomori methenamine silver or periodic acid Schiff stains (PAS) are used to detect fungal pathogens, if present. PAS, however, only works on living fungal organisms. Granulomas can also represent a response to foreign material, such as aluminum-based adjuvants or inoculated keratin.

One high-exposure frog (20C1) exhibited an amorphous eosinophilic (amyloid-like) material in the renal hematopoietic area adjacent to granulomas. Spawning salmon brains, presumably stressed, have been reported to have beta-amyloid deposits (Norris and Carr, 2013) detectable immunologically. Severe splenic amyloidosis has also been reported in an adult male captive spiny-tailed iguana, *Ctenosaura acanthura* (Goff et al., 1984). It is reasonable to hypothesize that frogs stressed by whatever elicited the robust granulomatous inflammatory response observed in these animals may also elicit deposition of amyloid-like substance at that site.

The severity (extent and number) of granulomas in high-dose frogs may have been slightly greater than that seen in control frogs, although the small sample size prohibits definitive statements. Biologically, the high-dose animals may have exhibited renal impairment due to disruption of tubule or hematopoietic function, which may have been less likely in control frogs. This suggests greater susceptibility by high-exposure animals than control frogs to the cause of the granulomatous response. Clinical data was not available for correlation with this study. A larger study including more animals may be useful to evaluate effect of NTO exposure on the innate or adaptive immune

response of *L pipiens.* Efforts to eliminate the source of the granulomatous response may help eliminate confounding pathology in this toxicity study.
No evidence of toxicity or developmental delay was observed in the examined tissues, suggesting NTO may not affect anuran renal or hepatic development. NTO, however, is known to adversely affect rat spermatogenesis, and some substances that do not affect mammals are more injurious to animals of other phyla; therefore, it may be informative to submit anuran reproductive or central nervous tissues for histologic examination.

7 Photomicrographs

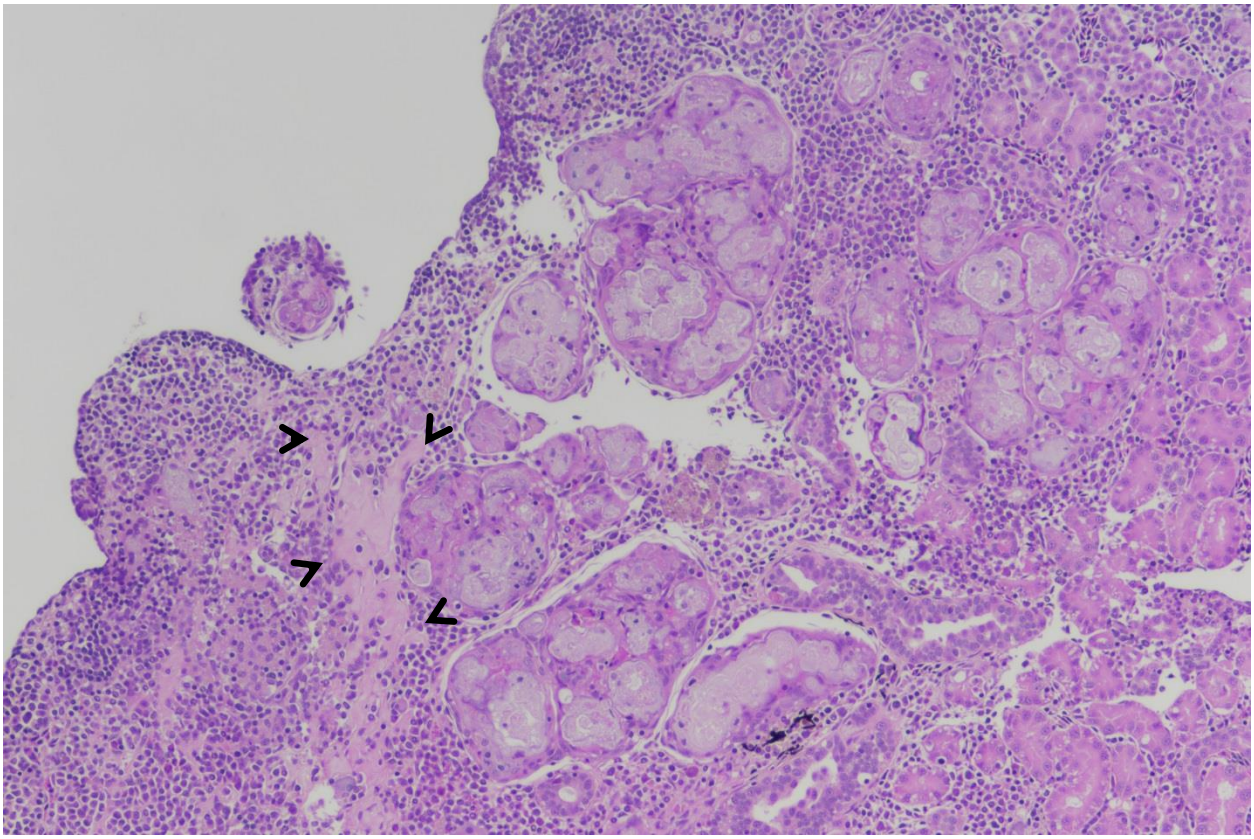

Figure 1. 20C1 High-dose Frog, Kidney

The subcapsular hematopoietic tissue is disrupted by numerous aggregates of macrophages encircling necrotic areas (granulomas). Pale eosinophilic amorphous material is also present (arrowheads) 10X

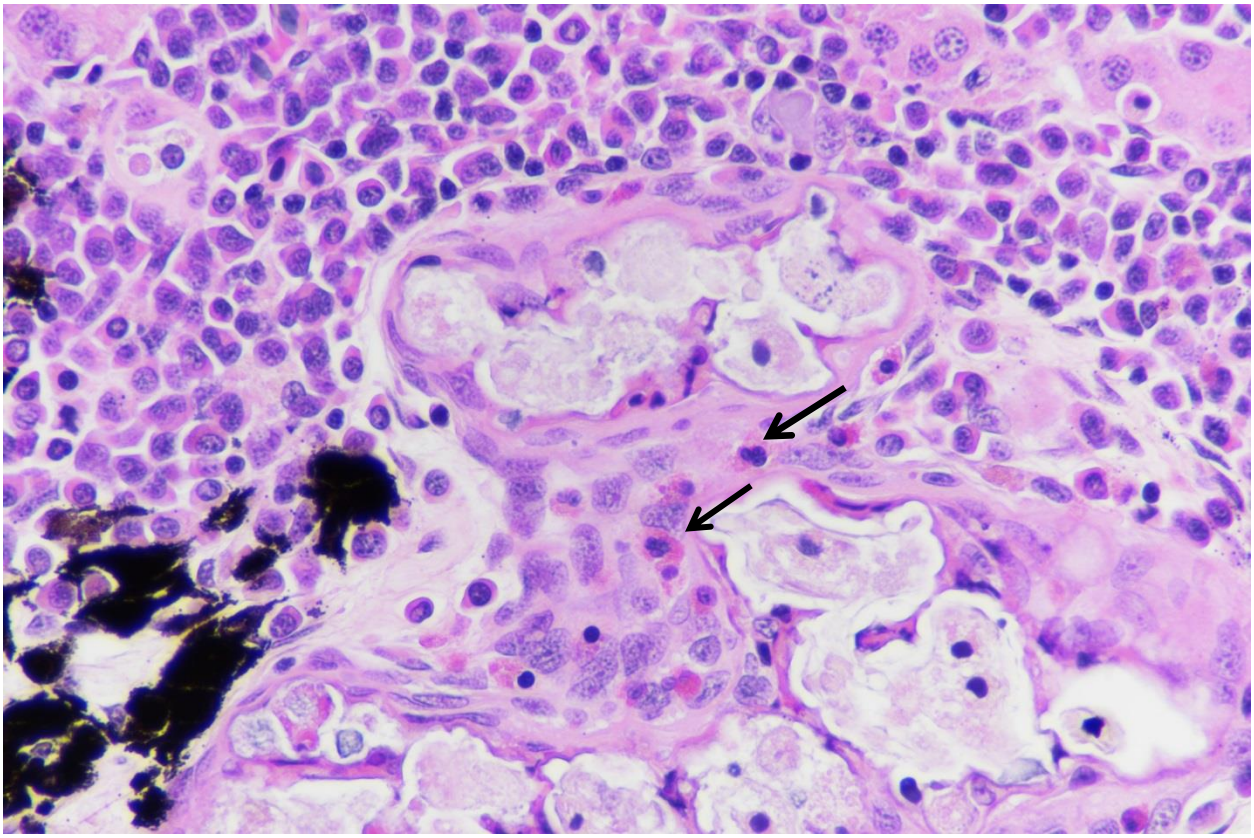

Figure 2. 00A1 Control, Kidney

Disrupting hematopoietic tissue in the kidney are multiple granulomas consisting of macrophages, some flattened, admixed with granulocytes with eosinophilic cytoplasmic granules (arrows) encircling necrotic cellular debris with pyknotic nuclei. 40X

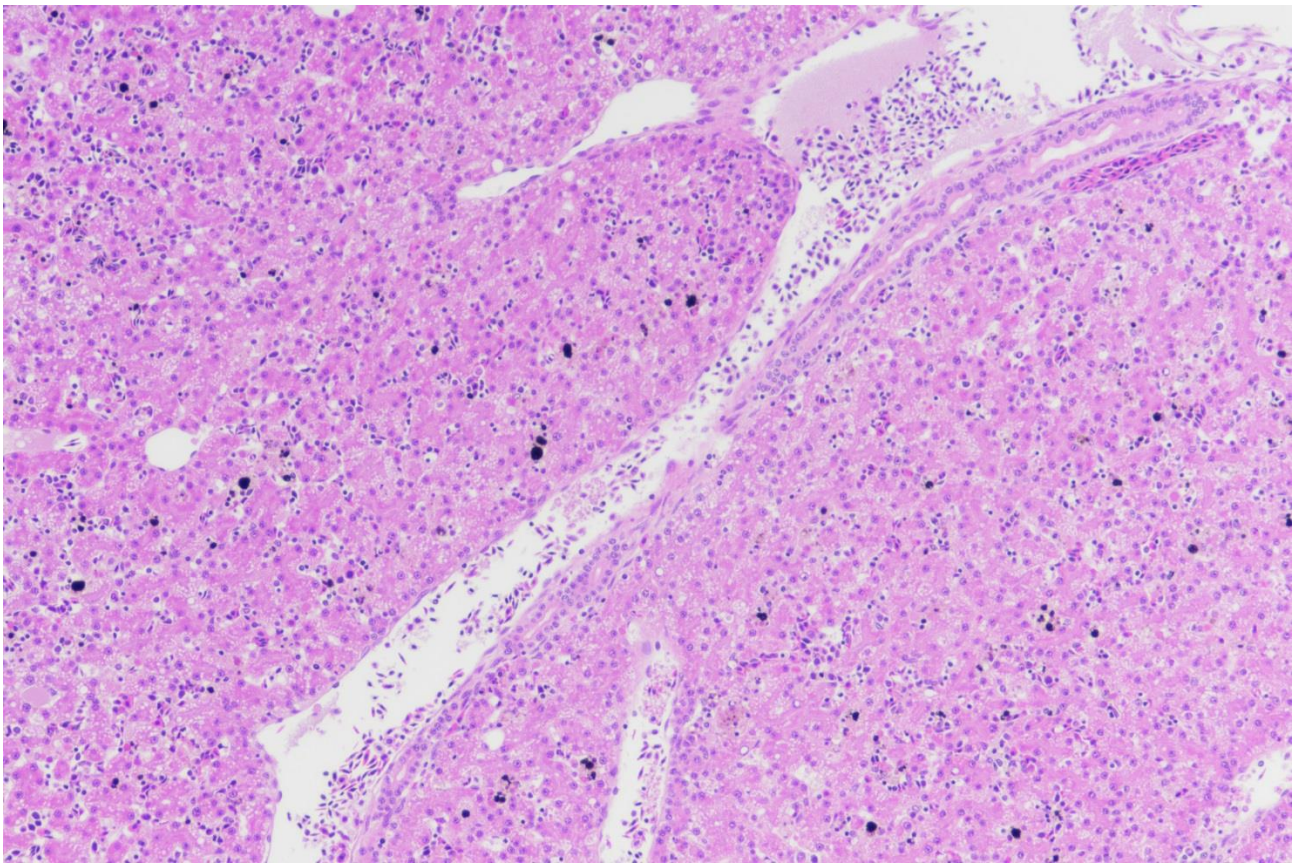

Figure 3. 20C1 High NTO-exposure Frog, Liver

Hepatocellular cords, sinuses central veins and bile ducts appear within normal limits. Pigment is commonly present in normal and NTO-exposed frogs. 10X

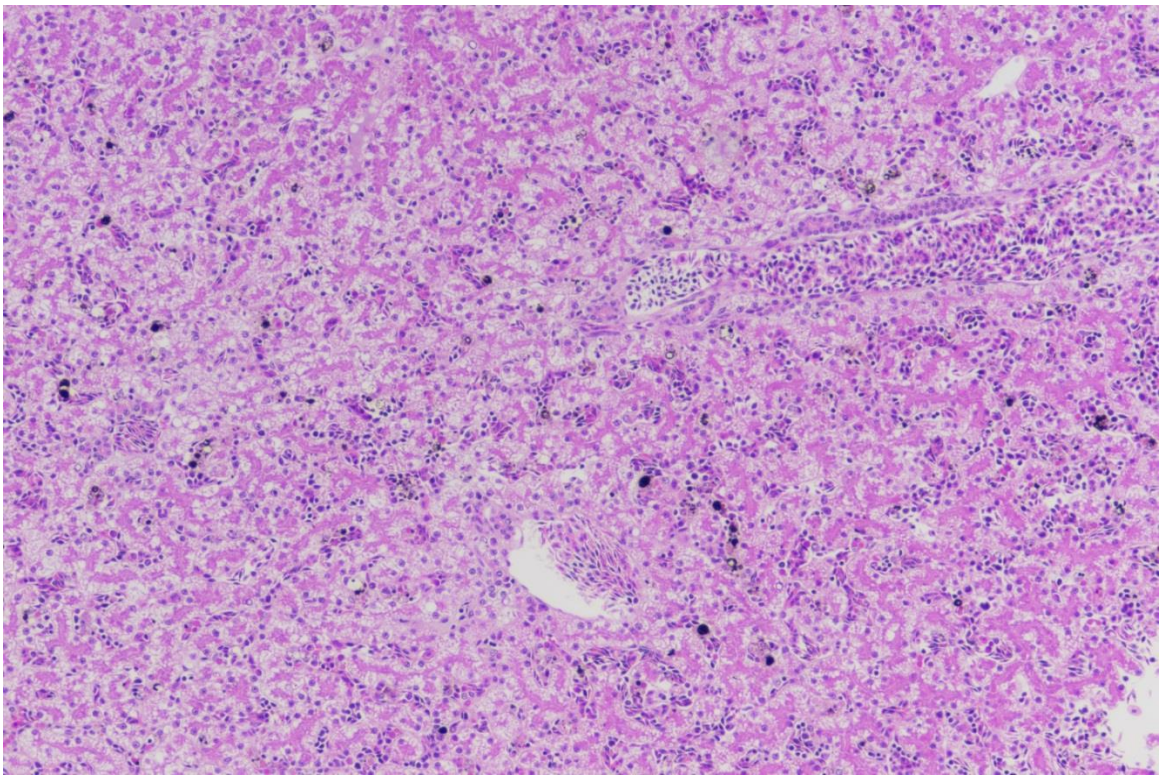

Figure 4. 00A1 Control Frog, Liver

Hepatocellular cords, sinuses central veins and bile ducts appear within normal limits. Pigment is commonly present in normal and NTO-exposed frogs. 10X

8 Point of Contact

The APHC (Prov) point of contact for this assessment is LTC Erica E. Carroll. She may be contacted at DSN 584-3980, commercial 410-436-3980, or via email at erica.e.carroll.mil@mail.mil.

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LTC, VC
Study Pathologist
Toxicology Directorate
Army Public Health Center (Provisional)

Approved:

MARK S. JOHNSON
Director, Toxicology

Appendix A

Quality Assurance Statement

The following critical phases were audited by the APHC (Prov) Quality Systems and Regulatory Compliance Office (QSARC), Laboratory and Toxicology Accreditation and Compliance Office (LTACO):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/ SD
Pathology Contributing Scientist Inspection - Quality Assurance Audit of Excel Entered Data	12/23/2015	12/24/2015
Pathology Contributing Scientist Inspection - Interim Pathology Report GLP Standard Regulation Review	12/23/2015	12/24/2015
Pathology Contributing Scientist Inspection - Final Pathology Report GLP Standard Regulation Review	12/24/2015	12/24/2015

Note 1. All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2. In addition to the study specific critical phase inspections listed here, general facility and process based inspections not specifically related to this study are done monthly or annually in accordance with QSARC, LTACO Standing Operating Procedures.

Note 3. This report has been audited by the Quality Assurance Unit (QSARC, LTACO) and is considered to be on accurate account of the data generated and of the procedures followed

Michael P. Kefauver
Quality Assurance Specialist, QSARC

24 December 2015
Date

Appendix B

Archives

B-1 Archives

All trim sheets, equipment logs, master data file (when present), and a copy of the final pathology report generated, as a result of providing pathology support, will be archived in the storage facilities of the Directorate of Toxicology, APHC (Prov), for a minimum of (10) years following submission of the final pathology report to the Sponsor.

A signed final pathology report is, for the purposes of Good Laboratory Practices, considered the raw data. The investigator will be provided a copy and the Army Public Health Center (Provisional) (APHC (Prov))-Toxicology (TOX) Portfolio will keep an electronic copy and one scanned copy to a computer disk. Scanned study files will be stored electronically in building E-2100, room 3010, APHC (Prov), Aberdeen Proving Ground, MD 21010. Any remaining wet tissue, paraffin blocks, and histology slides will be stored in building E-5158, unless the investigator requests them.

Pathology standing operating procedures are maintained by the Quality Assurance Unit in the Master Control database. Instrument maintenance logs are stored in room 1026 upon completion.

The archivist is Martha L. Thompson.

Appendix C

Addendum to Pathology Report

ADDENDUM TO

PATHOLOGY REPORT

For

One-Generation Study of the Effects of 3-nitro-1,2,4-triazol-5-one (NTO) to the Northern Leopard Frog (*Lithobates pipiens*) Under Static Renewal Test Conditions

TRE Project No.: 15001-468
Protocol No.: RP4OK.468.001

Study Director:
David Pillard, PhD

Prepared by:

LTC Erica Eggers Carroll, DVM, PhD, Diplomate, ACVP

Addendum to the Pathology Report for One-Generation Study of the Effects of 3-nitro-1,2,4-triazol-5-one (NTO) to the Northern Leopard Frog (*Lithobates pipiens*) Under Static Renewal Test Conditions

1 Background

Approximately 60 days after completion of the first phase of this project, a second shipment was received by Army Public Health Center (Provisional) (APHC (Prov)) Toxicology Directorate, Division of Toxicologic Pathology. Formalin-fixed testes from eight Northern Leopard frogs (*Lithobates pipiens)* were collected when the animals completed metamorphosis (Gosner Stage 46), and were humanely euthanized. Upon receipt, due to the small size of specimens, they were "sandwiched" between histology cassette sponges and processed using a biopsy setting on the tissue processor. Tissues were embedded, sectioned (2 micrometers (um) to maximize resolution for this study) and stained (with periodic acid Schiff [PAS] in the event spermatozoa were developing). In spite of extraordinary care in processing, of the eight animal tissues submitted, tissues from only five frogs (two controls and three "high concentration") were successfully processed onto microscope slides.

2 References

Papoulias D.M., M.S. Schwarz, and L. Mena. 2013. Gonadal abnormalities in frogs (Lithobates spp.) collected from managed wetlands in an agricultural region of Nebraska, USA. *Environ Pollut*. 172:1-8.

Haczkiewicz K. and M. Ogielska. 2013. Gonadal sex differentiation in frogs: how testes become shorter than ovaries. *Zoolog Sci*. 30(2):125-134.

Table 1. Summary of Testicular Samples Collected from Male *Lithobates pipiens*

Sample ID	Organ/ Tissue	Treatment	~NTO Concn. (mg/L)	Replicate	Specimen #	Comment – Histological Evaluation
00C2-TES	Testes	Control	0	C	2	2 testes collected
00D1-TES	Testes	Control	0	D	1	1 testis collected – n/a
00D2-TES	Testes	Control	0	D	2	1 testis collected – n/a
00D4-TES	Testes	Control	0	D	4	2 testes collected
20B2-TES	Testes	High Concn.	3300	B	2	Likely male; 1 testis collected
20B3-TES	Testes	High Concn.	3300	B	3	Likely male, 1 testis collected - n/a
20D1-TES	Testes	High Concn.	3300	D	1	2 testes collected
20D2-TES	Testes	High Concn.	3300	D	2	2 testes collected

Note:
n/a = not available for evaluation; tissue lost during processing

3 Results

- 00C2 Control testis: There are some distinct seminiferous tubules, numerous spermatogonia, with 1-2 prominent nucleoli, often indented or kidney bean-shaped nuclei. Mitotic figures or binucleate cells average 1–2/40x field. Vacuoles commonly disrupt the spermatogonia cytoplasm. Scant black pigment is just beneath the tissue surface (Figure 1).

- 00D4 Control testis: Seminiferous tubules are not evident. Spermatogonia are numerous with prominent chromatin. Some nuclei have 1–2 distinct nucleoli. Nuclei are occasionally offset by 1–2 cytoplasmic vacuoles. There is scant subsurface black pigment (Figure 2).

- 20B2 High-concentration NTO testis: There are distinct seminiferous tubules with possibly fewer spermatogonia (3–4 per tubule instead of 5 or more per tubule as seen in the two controls. Chromatin is less prominent than in 00D4 but there are 1–2 distinct nucleoli. There is scant superficial pigment (Figure 3).

- 20D1 High-concentration NTO testis: Distinct seminiferous tubules are at the margins of the section with densely packed smaller, darker cells in the center (fibroblasts and early Leydig cells). Spermatogonia chromatin is less distinct than in 00D4 (control). Binucleate, indented and kidney-bean shaped nuclei are common (Figure 4).

- 2D02 High-concentration NTO testis: Seminiferous tubules appear smaller than in controls, contain fewer spermatogonia which have indistinct chromatin. Binucleate spermatogonia are common, each nucleus with one distinct nucleolus (Figure 5).

4 Discussion

Figures 1 and 2 demonstrate normal inter-individual variation in testicular development. Although collected at Gosner stage 46, upon completion of metamorphosis, formation of seminiferous tubules varied among control frogs with some exhibiting apparently randomly scattered spermatogonia and precursors to Sertoli cells, interpreted as less mature (00D4). Other specimens exhibited clearly formed tubules with Sertoli cells arranged in support of Spermatogonia, some with mitotic figures (00C2). A similar range of maturity was present in the NTO-exposed anurans (Figures 3–5). Black pigment is normal in several frog tissues. The effect of NTO exposure is unclear and one cannot make definitive statements upon evaluating five animals but some sections have fewer, less robust spermatogonia than others; it remains to be determined if this occurs more often in NTO-exposed animals compared to control frogs. As tissues were collected prior to the development of mature spermatozoa, spermatogenesis could not be evaluated.

Tissues from three frogs were lost during processing of eight submitted tissues. At this stage of development *L. pipiens* testes are so small (often < 3 millimeters (mm) in any dimension) as to be extremely challenging to collect and process with optimal results. We suggest, henceforth, for structures of interest measuring less than 4 mm in any dimension, that transverse sections of the entire animal be submitted, no thicker than 4 mm thick. This method will help preserve tissue architecture.

5 Photomicrographs

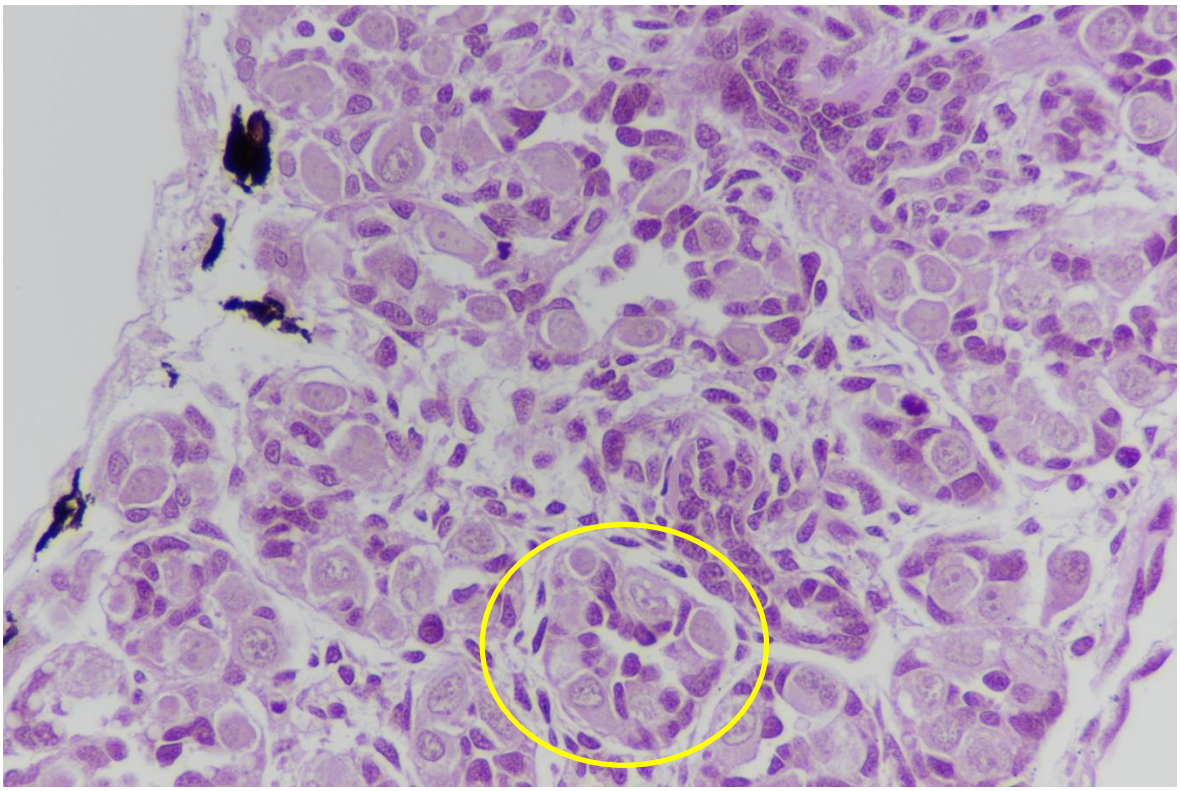

Figure 1. Control Frog (00C2) Testis

In this section seminiferous tubules are more mature as spermatogonia and early Sertoli cells form recognizable seminiferous tubules (outlined). 40X PAS

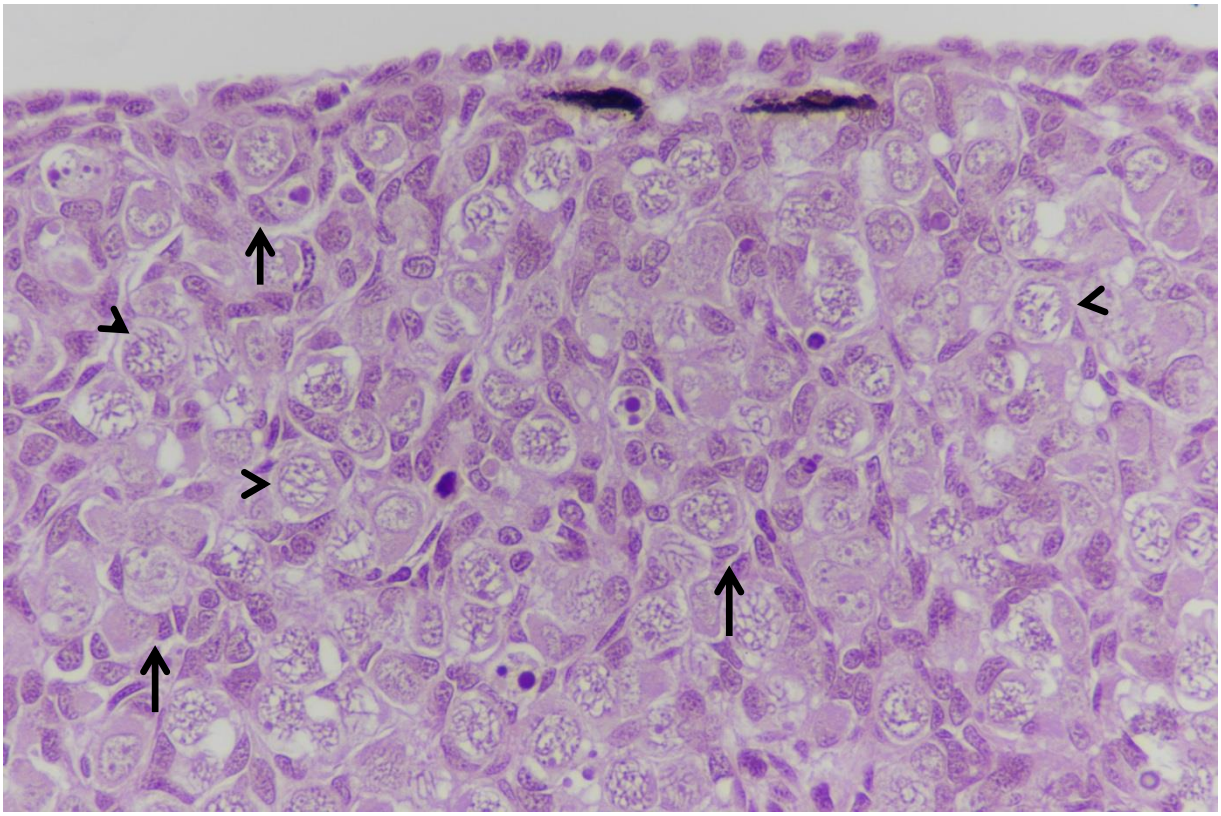

Figure 2. Control Frog (00D4) Testis

Gosner stage 46 (per contributor) In this section seminiferous tubules are immature and therefore indistinct. Robust spermatogonia (arrowheads) are interspersed among smaller, darker precursors of Sertoli cells (arrows). 40X PAS

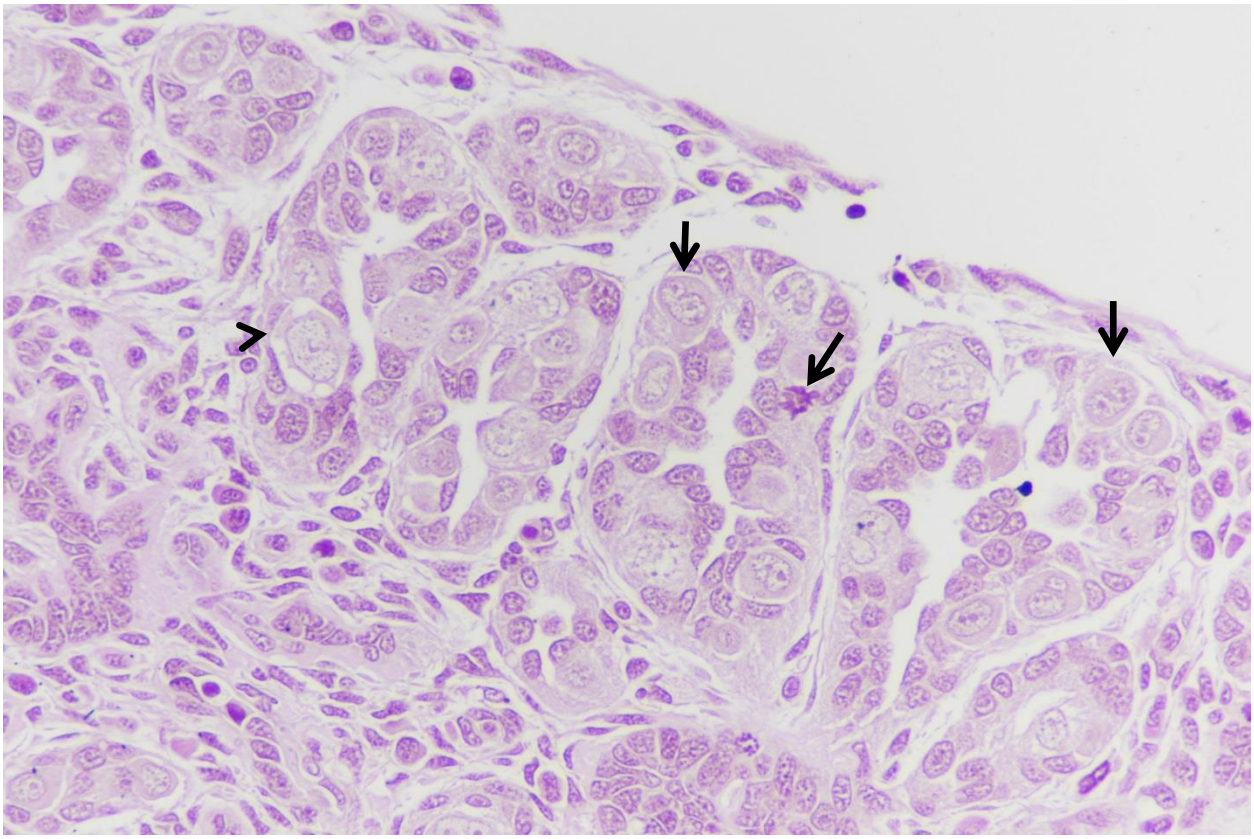

Figure 3. High-Concentration NTO Frog (2D01) Testis

Seminiferous tubules at the margin of the testis with smaller cells in the center of the tissue. Spermatogonia occasionally have vacuolated cytoplasm (arrowhead) and deeply indented nuclei or mitotic figures (arrows) 40X PAS

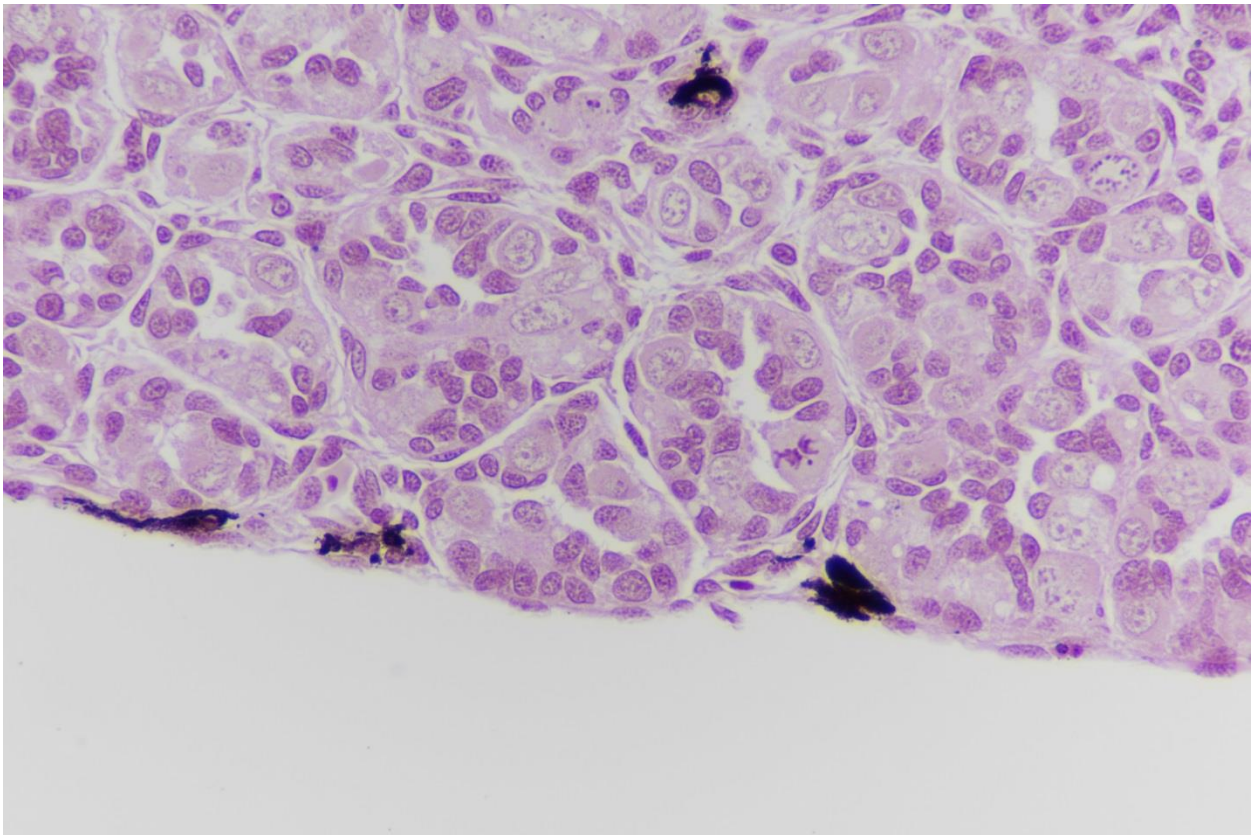

Figure 4. High-Exposure Frog (20B2) Testis

Seminiferous tubules are distinct with occasional mitotic figures of spermatogonia. Pigment is present, general at the tissue surface. 40X PAS

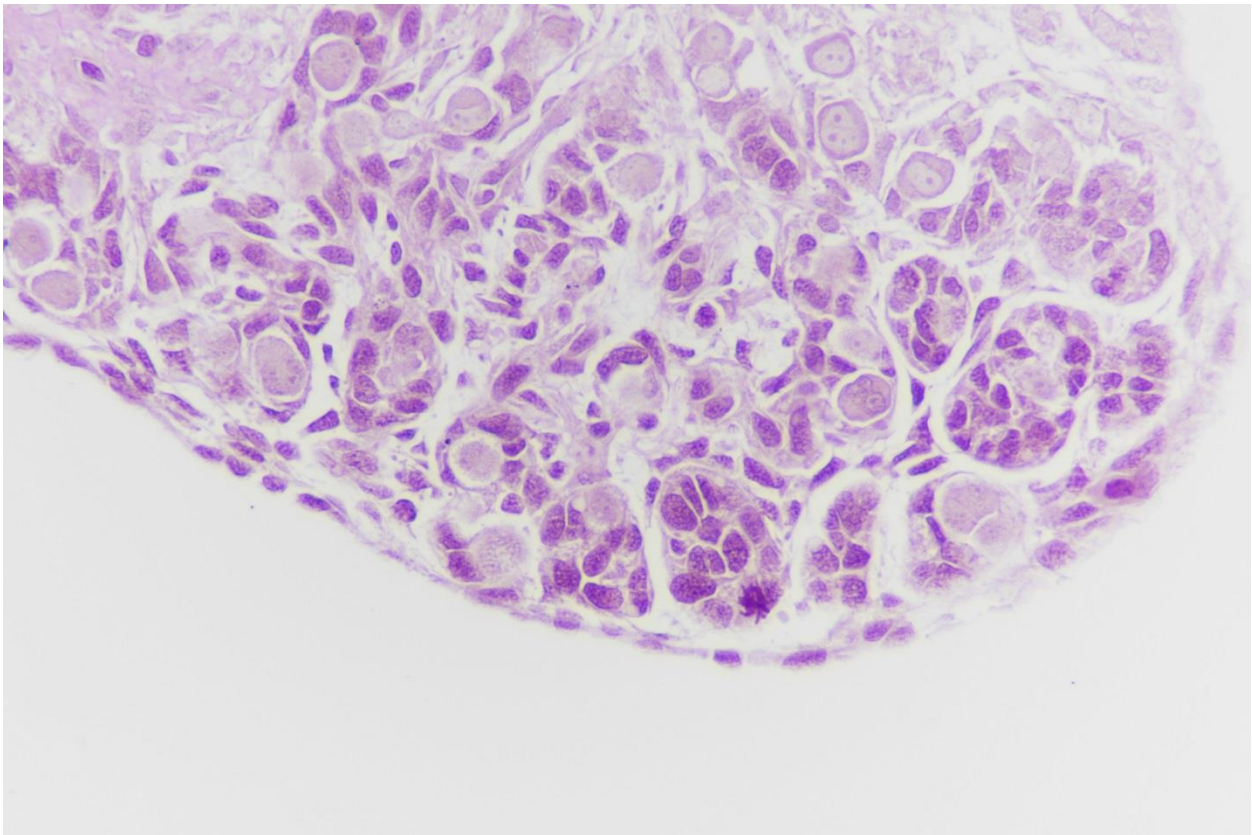

Figure 5. High-Concentration NTO Frog Testis

(20D2) This specimen has smaller seminiferous tubules, less robust Spermatogonia. 40X PAS.