Exhibit 28

Exhibit 28

November 29, 2017

DRAFT

Development of a Water Effect Ratio for Nickel in the Sangamon River

Prepared by Windward Environmental, LLC
for the Sanitary District of Decatur

Windward environmental LLC

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

This report was prepared in support of the Sanitary District of Decatur's ("District") Petition to the Illinois Pollution Control Board ("Board") seeking a Site-Specific Rule to establish an alternative water quality standard ("WQS") for Nickel ("Ni") from the point of its discharge into the Sangamon River from its Main Sewage Treatment Plant ("Main Plant") to the point of the confluence of the Sangamon River with the South Fork of the Sangamon River near Riverton, Illinois. The purpose of this report is to document the development of a site-specific water quality standard for Ni for the Sangamon River to account for bioavailability effects using a Water Effect Ratio ("WER").

WATER EFFECT RATIO CALCULATION

Rationale for WER adjustment

Many factors can modify the bioavailability and toxicity of Ni, including hardness and natural organic matter (NOM). The Sangamon River chemistry is hard water with considerable amounts of organic matter. The Illinois Ni standard is based on hardness, so hardness effects are already addressed. However, the state standard does not consider ameliorative effects of NOM on Ni. The WER is an approach developed by US EPA to develop a site-specific standard that can account for toxicity modifying factors that affect the bioavailability of metals that are not otherwise addressed by the state-wide standard. We have developed a WER for the Sangamon River which was derived to consider NOM. The WER, together with the hardness equation, will define a site-specific standard that incorporates the effects of both NOM and hardness, which are the two primary factors that affect Ni bioavailability and toxicity.

NOM effects on Ni toxicity

Natural organic matter has been shown to reduce the bioavailability and toxicity of Ni (Hoang, Tomasso et al. 2004, Kozlova, Wood et al. 2009). The effects of NOM are one of the primary reasons why a site-specific adjustment to the Ni standard is justified. The effects of NOM on Ni bioavailability were confirmed by chronic *C. dubia* toxicity tests performed at Oregon State University (OSU). OSU conducted these Ni toxicity tests to support the development of a WER for Ni in the Sangamon River (OSU 2017, OSU 2017). For these tests, the exposure conditions were designed to match the ionic composition of the Sangamon River with and without added NOM. Chronic toxicity tests with *C. dubia* were conducted to quantify Ni toxicity on survival and reproduction.

The results of the OSU tests confirm that DOC reduces Ni bioavailability and toxicity (OSU 2017, OSU 2017).

Determination of a DOC equation

The response in the OSU data confirms information found in the literature about the reduction in Ni toxicity due to the presence of NOM. Dissolved organic carbon (DOC) is an analytical measurement used to quantify NOM, although the two terms are frequently used interchangeably. These data were used to develop a general DOC relationship. To develop this relationship, data from the OSU tests were combined with data from the literature. In considering literature data, the most relevant data would be for a sensitive organism that also exhibits a response to DOC comparable to the response seen for *C. dubia* used in the OSU tests. The most comparable literature data are the D. pulex study by Kozlova et al (2009). The OSU and Kozlova et al data were used in an ANCOVA analysis to develop an overall DOC equation for the Sangamon River (Appendix 2). The resulting equation is:

$$\log_{10} Ni = 0.329 * \log_{10} DOC + 0.919$$

This equation can be used to calculate Ni effects as a function of DOC in site water or reference waters by simply using appropriate DOC concentrations for each of these waters. The DOC in the reference water tests used in the OSU study was reported as 0.5 mg/L. The DOC concentrations in the Sangamon were quantified in samples taken downstream of the Main Plant. These data can be used to calculate a WER as follows:

$$WER = \frac{Ni\ effect\ in\ site\ water}{Ni\ effect\ in\ reference\ water}$$

Where the Ni effects are calculated using the DOC equation derived from the ANCOVA analysis (Appendix 2).

Calculation of a WER for the Sangamon River

The DOC equation can be used with measured DOC concentrations in the Sangamon to calculate a WER for the Sangamon River. Monitoring samples taken from downstream of the Main Plant were previously characterized (Santore, 2015). These data result in an average DOC concentration of 7.91 mg/L. Substituted these DOC values in the WER equation yields a WER value of 2.48.

SUPPORTING INFORMATION FOR THE NI WER USING THE NI BLM

The Biotic Ligand Model (BLM) is a predictive model that can also be used to account for Ni bioavailability. The BLM has been adopted by US EPA for determining the water quality criteria for copper (USEPA 2007). The BLM for Ni has been evaluated against a large number of toxicity datasets (Santore et al., in prep). The Ni BLM has been used to estimate a WER for the Sangamon River (Santore 2014). The estimated WER using the Ni BLM is 2.6, which is in excellent agreement with the WER derived from the OSU toxicity tests.

SUMMARY AND CONCLUSIONS

Chemical factors in receiving waters can modify the toxicity of metals to aquatic organisms. In the Sangamon River, the effects of both hardness and NOM are important toxicity modifying factors that can affect Ni toxicity. The Illinois Ni standard considers hardness, but does not consider the effects of NOM. The effects of NOM were quantified for the Sangamon using chronic toxicity tests with *C. dubia,* which is the most sensitive aquatic organism in the Illinois state standard. The toxicity tests indicate that NOM is protective against Ni toxicity for aquatic organisms in Sangamon. The effect of NOM on Ni toxicity was quantified by relating measured toxicity to DOC, which is a measure of NOM quantities in the Sangamon. The quantification of NOM effects were based on an ANCOVA analysis of data from this study as well as relevant toxicity data from the scientific literature. The resulting equation was then used with downstream monitoring data to determine an overall equation that relates Ni toxicity to measured DOC concentrations. This equation, applied to average DOC concentrations in the Sangamon results in a WER of 2.48. This WER was corroborated by an independent analysis using the Biotic Ligand Model. The BLM, applied to conditions in the Sangamon generates an almost identical WER of 2.6. The excellent agreement between these results provides supporting evidence that the WER of 2.48 is a reasonable and defensible result for the Sangamon River.

APPENDIX 1 – OSU NI TOXICITY TEST

Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*

Prepared for

SANITARY DISTRICT OF DECATUR
501 Dipper Lane
Decatur, IL 62522
USA

Prepared by

OREGON STATE UNIVERSITY
DEPARTMENT OF ENVIRONMENTAL AND MOLECULAR TOXICOLOGY
AQUATIC TOXICOLOGY LABORATORY
33972 Texas St. SW
Albany, OR 97321
USA
T. 541.926.1254

CITATION

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cladoceran, *Ceriodaphnia dubia*.
Prepared for the Sanitary District of Decatur.
May 2017.

ABSTRACT

In an effort to determine the chronic toxicity of nickel (Ni) in a simulated effluent, with and without the addition of dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*, a series of chronic toxicity tests were performed. Previously conducted toxicity tests, as part of a water-effect ratio (WER) project, were performed with Ni exposures in a simulated effluent and a site effluent from the Sanitary District of Decatur (SDD, Decatur, IL, USA) for determining differences in Ni toxicity between the two waters. In the previous studies, DOC was not added to the simulated effluent, but the site effluent contained approximately 15 mg/L DOC, therefore a comparison between waters was difficult due to the differences in DOC concentrations. The tests reported here were conducted to compare the effects of Ni exposure in a simulated effluent both with and without added DOC. DOC was added (as Suwannee River Isolate) at a concentration (nominal 14 mg/L) comparable to that observed in the previously tested site effluent. The simulated effluent was also prepared to match the high cationic and anionic parameters (calcium, magnesium, sodium, potassium, sulfate, and chloride) of the previously tested site effluent. Testing followed the standard USEPA short-term chronic toxicity testing methodology. Test endpoints included an assessment of survival and reproduction. In both tests, an exposure:response relationship was observed for both survival and reproduction based upon the nickel exposure concentrations. Survival and reproductive endpoints are presented in the table below. The current study and the previous study, with the simulated effluent without DOC, had very similar outcomes. In the present study, Ni toxicity was reduced in the simulated effluent with added DOC. In addition, the nickel biotic ligand model (BLM) was used to predict toxicity values versus the observed results. The input values for a range of DOC concentrations (those measured in the waters without food and those measured after 24 hr exposure with food) were used and the range of predictions is shown below. The BLM-predicted effect concentrations and the observed effect concentrations for the simulated effluent without added DOC were very close, while the BLM over-predicted the protective effect of DOC (i.e., DOC did not provide as great of a protective effect as the model predicted, by approximately a factor of two).

Test water		**Survival**			**Reproduction**		
		NOEC	**LOEC**	**EC_{20}**	**NOEC**	**LOEC**	**EC_{20}**
		µg/L dissolved Ni					
Present study	Simulated effluent **without** added DOC	8.0	10.8	8.3 (7.3 – 9.4)	8.0	> 8.0	8.0 (6.1 – 10.6)
	BLM prediction *	-	-	8.7 – 11.5	-	-	4.8 – 6.4
	Simulated effluent **with** added DOC	23.7	32.5	26.3 (23.6 – 29.3)	12.3	17.4	16.1 (14.6 – 17.7)
	BLM prediction **	-	-	60.9 – 71.2	-	-	34. 8 – 40.8
Previous study	Simulated effluent **without** added DOC	12.6	18.2	13.0 (11.8 – 14.3)	4.7	6.4	7.4 (5.2 – 10.5)
	BLM prediction	-	-	13.77	-	-	7.24

* BLM prediction is based upon a range of DOC concentrations (low value of 0.10 mg/L to high value of 0.98 mg/L)
** BLM prediction is based upon a range of DOC concentrations (low value of 11.3 mg/L to high value of 13.6 mg/L)

TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

SIGNATURE PAGE

Sponsor:	Sanitary District of Decatur 501 Dipper Lane Decatur, Illinois 62522 USA
Title:	Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*
Testing Facility:	Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox) 33972 Texas St. SW Albany, Oregon 97321 USA

Principal Investigator Signature: ________________________ ________________
William Stubblefield, Ph.D. Date

Study Director Signature: ________________________ ________________
Allison Cardwell Date

INDIVIDUAL TEST PERSONNEL PAGE

Sponsor:	Sanitary District of Decatur 501 Dipper Lane Decatur, Illinois 62522 USA
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The names of key OSU AquaTox scientists, professionals and/or supervisory personnel who participated in this study include:

OSU AquaTox Scientists:	Jesse Muratli, Toni Hoyman, Matt Sroufe, Emily Stefansson
Principal Investigator:	William Stubblefield, Ph.D.
Study Director:	Allison Cardwell

QUALITY ASSURANCE STATEMENT

The conduct of the study, "Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*" was reviewed for compliance with the test protocol and OSU AquaTox Standard Operating Procedures (SOPs). Testing and documentation for the study were carried out in the spirit of U.S. EPA Good Laboratory Practice (GLP) standards.

Principal Investigator Signature: ________________________ ________________
William Stubblefield, Ph.D. Date

Study Director Signature: ________________________ ________________
Allison Cardwell Date

1. INTRODUCTION

The testing reported herein was conducted to determine the toxicity of nickel (Ni) to the cladoceran, *Ceriodaphnia dubia*, when exposed in a laboratory-reconstituted water designed to simulate an effluent collected from the wastewater treatment facility in Decatur, IL. Tests were conducted both with and without the addition of dissolved organic carbon (DOC). The studies were conducted as 7-day chronic toxicity tests according to standard USEPA testing methodology (USEPA 2002). To determine chronic toxicity, survival and reproduction were assessed during the 7 day test period.

Due to the complex ionic makeup of the simulated effluent, the test organisms were acclimated for over a year to the high ionic composition of the water. Following many months of acclimation, starting from a very hard reconstituted water culture, the *C. dubia* cultures have been maintained successfully in the simulated effluent.

Testing and documentation for the study were carried out in the spirit of Good Laboratory Practice (GLP) standards. The study was conducted at the Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox, Albany, OR, USA). Chemical analysis of the metals was performed at the OSU W.M. Keck Plasma Spectrometry Laboratory (Corvallis, OR, USA). Chemical analyses of the dilution water were performed at CH2M Hill (Corvallis, OR, USA). All data related to this study will be maintained in the OSU AquaTox archives for 10 years.

2. MATERIALS AND METHODS

2.1 METHODOLOGY

The studies were conducted according to the protocol, "Short-term chronic nickel toxicity in simulated effluent laboratory water with and without dissolved organic carbon (DOC) to the cladoceran, *Ceriodaphnia dubia*, under static-renewal test conditions" OSU AquaTox Protocol No. NIC-CD-CSR7d-005, effective in November 2016. Appendix A (Protocol) contains a copy of the protocol. The studies were conducted according to standard USEPA methodology (USEPA 2002).

2.2 TEST SUBSTANCE

The test substance, reagent grade nickel chloride hexahydrate ($NiCl_2$ x $6H_2O$; CAS number 7791-20-0, Lot #L05582) was received from J.T. Baker (Avantor Performance; Phillipsburg, NJ, USA). The test substance had a reported assay purity of 100.0%. The certificate of analysis is provided in the report appendices. The manufacturer's material safety data sheet reported a solubility in water of 2,540 g/L at 20°C. Following receipt at OSU AquaTox, the test substance was stored sealed in its original container at room temperature.

A stock solution of 20 mg/L nominal Ni was prepared by addition of $NiCl_2$ x $6H_2O$ to Milli-Q water in a volumetric flask, followed by storage in a plastic container in the dark at 0-6°C. All nickel concentrations are expressed as micrograms Ni per liter (µg/L Ni).

2.3 DISSOLVED ORGANIC CARBON

DOC was added to the control/dilution water (Section 2.5/2.6) of the simulated effluent with added DOC test in the form of Suwannee River Natural Organic Matter (NOM; Product R101N obtained from International Humic Substances Society, St. Paul, MN, USA). The NOM was added to achieve a nominal DOC concentration of 14 mg/L, based upon a 48% carbon composition in the NOM.

2.4 TEST SYSTEM

2.4.1 SPECIES

The freshwater cladoceran, *Ceriodaphnia dubia*, was used in testing.

2.4.2 SOURCE

The tests were initiated with <24 hour old neonates from an in-house culture (see culture acclimation in Section 2.4.3). Parental organisms were isolated onto brood boards in order to obtain <24 hr old neonates for testing.

2.4.3 CULTURE ACCLIMATION

C. dubia were obtained from an in-house culture that was acclimated and successfully cultured in laboratory water that was diluted (with deionized water [Milli-Q®]) from a water designed to simulate the ionic composition of the Decatur full-strength effluent (nominal water quality parameters described in Table 2-1) for over a year. During the initial acclimation period, and as discussed in a previous report (OSU 2016), *C. dubia* adults were slowly acclimated to undiluted simulated effluent over the course of 6 weeks and cultured for a period of 6 weeks in the undiluted simulated effluent. Culture reproduction varied over time and therefore a 20% dilution of the full-strength simulated effluent was employed (80:20 simulated effluent:deionized water [Milli-Q®]) to remove the potential of any toxicity due to the high ionic content of the water. During the course of culturing in the diluted simulated effluent, survival and reproduction were excellent and organism health was maintained over a period of over 1 year.

2.5 DILUTION WATER

The control/dilution water for the simulated effluent tests consisted of a laboratory water made from deionized water amended with the appropriate reagent grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$) that was diluted by 20% with deionized water (80% simulated water:20% deionized water). Before dilution, the simulated effluent was prepared to achieve nominal concentrations detailed in Table 2-1. Preparation steps are detailed in Section 2.5.1. Each water was amended with trace amounts of vitamin B_{12} and Se, as per USEPA (2002) methodology for *C. dubia* culture and testing.

Table 2-1. Target simulated effluent water quality before and after dilution

Water	Hard.	Alk.	Ca	Mg	Na	K	Cl^-	SO_4	pH
	(mg/L as $CaCO_3$)		mg/L						SU
Simulated Effluent before dilution	400	400	52.2	56.5	467.3	102.2	423.5	348.5	8.3
Simulated Effluent after dilution	324	436	52.5	45.9	348.0	81.3	315	295	8.5

SU = Standard Units.

2.5.1 SIMULATED EFFLUENT/LABORATORY WATER PREPARATION

Dilution water was prepared as follows:

- Addition of reagent grade salt ($CaSO_4 \cdot 2H_2O$) to deionized water and mixed overnight.
- Addition of reagent grade salt ($MgSO_4$) to deionized water and mixed overnight.
- Addition of reagent grade salts (KCl, NaCl, and $NaHCO_3$) to deionized water and mixed overnight.
- Solutions combined and mixed.
- Solution bubbled with CO_2 to reduce pH and promote carbonate dissolution. pH reduced to below 6.0. Water left in a zero headspace container overnight.
- Solution bubbled with O_2 to increase pH to above 8.0.
- Solution diluted by 20% with deionized water.
- Light aeration for the duration of the test.
- The water was split into two aliquots to which Suwannee River Isolate (DOC) was added to achieve a nominal target DOC concentration of 14 mg/L. Because of issues with control acceptability criteria (details provided in Section 3.1) in the initial “without DOC” simulated

effluent test, a second batch of the simulated effluent was prepared, as above, and that test was re-conducted and is reported here. The original "with DOC" test was not re-conducted and the original results are reported here.

2.6 ROUTE OF EXPOSURE AND SELECTION OF TEST CONCENTRATIONS

Method: Appropriate volumes of nickel stock were added individually to each dilution water (see Section 2.5) to achieve intended nominal concentrations. Following the spiking of nickel to each concentration, the solutions were equilibrated, at test temperature, for 3 hours prior to use.

Frequency: A 100% renewal of control and treatment solutions occurred daily by transferring each original adult organism to a freshly prepared exposure chamber. Each day, prior to organism transfer, solutions were equilibrated 3 hours prior to use.

For each test (simulated effluent/laboratory water with and without DOC), seven test treatments and a control were tested using a 0.7 dilution scheme (i.e., exposure concentrations were 70% of the preceding concentration). The selection of nominal test concentrations was based upon previously conducted studies exposing *C. dubia* to nickel-spiked simulated effluents (OSU 2016), chronic toxicity of nickel from biotic ligand model (BLM) predictions, and early range-finding screening. Each test concentration (treatment) was prepared in a batch and then distributed to the test chambers. Ten replicate chambers were prepared for each concentration and control.

One additional treatment of concurrent very hard reconstituted control water (VHW RW; nominal hardness/alkalinity of 315/229 mg/L as $CaCO_3$) (USEPA 2002) was included with the simulated effluent without DOC test.

2.7 TEST CHAMBERS

Organisms were exposed in new 30 mL polypropylene Soufflé (Solo® Brand, Canada) cups containing approximately 25 mL of test solution.

2.8 TEST CONDITIONS

The test chambers were housed in a temperature-controlled environmental chamber designed to maintain the test temperature at 25 ± 2 °C. The test was conducted under a 16:8 hour light:dark cycle using cool-white fluorescent lights at ~100 foot candles. The test chambers were randomized based upon a computer-generated randomization scheme.

2.9 TEST INITIATION, RENEWAL, AND FEEDING

To initiate the tests, neonates (< 24 hrs old) from a single adult from the acclimated culture were distributed into one row (1 neonate for 1 replicate of each treatment) of randomly ordered test chambers. This process was repeated using a new brood of neonates from a single adult for each row of the entire randomization pattern to initiate testing. Each test chamber was fed 0.3 mL of an algae (*Pseudokirchneriella subcapitata*) and yeast/trout chow/cereal leaf (YTC) suspension (1:1) at test initiation (prior to introduction of the test organism) and once daily prior to water renewal. On a daily basis, only the original organism was transferred to a freshly prepared test chamber and neonates were counted daily.

2.10 TEST MONITORING

2.10.1 WATER QUALITY

Temperature, pH, conductivity, total dissolved solids (TDS), and dissolved oxygen (DO) were measured in each concentration at test initiation, once daily throughout the test, and at test termination. These parameters were measured both in "new" waters (solutions prior to daily use) and in "old" waters (solutions sampled directly from the test chamber [a composite of each replicate of each concentration per day]). Hardness, alkalinity, ammonia, and total residual chlorine (TRC) were measured in the control water of each test at test initiation. Hardness and alkalinity were also measured on Days 3 and 6 in the control, one middle Ni exposure, and one high Ni exposure in both new and old waters. Temperature was measured with a standard laboratory thermometer. Test solution pH was measured using a HACH (Loveland, CO, USA) HQ30d pH meter. Conductivity and TDS were measured using a HACH Sension5 meter. Dissolved oxygen was measured using a HACH HQ10 meter. Ammonia was measured using a HACH HQ40d meter. TRC was measured with a HACH2 Pocket Colorimeter II. Water hardness and alkalinity were measured by colorimetric titration (Standard Methods 2340B/C and 2320B [APHA 2012]).

Certain water quality parameters were measured at an outside commercial laboratory (CH2M Hill, Corvallis, OR, USA). Calcium, magnesium, sodium, potassium were measured via Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; EPA Method 200.7) (USEPA 1994a). Chloride and sulfate were measured via Ion Chromatography (EPA Method 300.0, USEPA 1993) and dissolved organic carbon (DOC) was measured via Combustion (Standard Methods 5310B; APHA 1998). The cations and anions were measured in new waters and the DOC was measured in both new and old waters from the toxicity tests.

2.10.2 BIOLOGICAL MONITORING

Observations of live and dead organisms were conducted on a daily basis from initiation to termination. The number of young was counted daily. Only original live adult organisms were moved to fresh solution daily.

2.10.3 ANALYTICAL SAMPLING

Total Metals

Analytical samples from each treatment were collected for total Ni analysis from newly prepared waters ("new" waters, following equilibrium periods and just prior to use) at test initiation, and on Days 3 and 6 of each test. Samples from old waters (a composite sample of each replicate within a treatment) were collected on Day 4 and at test termination. The samples were collected by drawing 5 mL of solution into a syringe to rinse the inside of the syringe and then disposing of the solution. Next, 15 mL of sample was drawn into the syringe and injected into a 15 mL polypropylene conical tube. Samples were preserved with trace metal grade nitric acid (AR-ACS grade, Mallinckrodt Chemical, Hazelwood, MO, USA) to $pH < 2$ and refrigerated (0 - 4 °C) prior to analysis.

Dissolved Metals

Analytical samples from each treatment were collected for dissolved (filtered through a 0.45 µm Acrodisc Supor PES filter, [Pall Life Sciences; Ann Arbor, MI, USA]) Ni analysis according to the same schedule as the total metals. Sampling occurred by drawing approximately 20 mL into the syringe of which 5 mL was pushed through the filter for disposal and the remaining 15 mL was collected into a 15-mL polypropylene conical test tube. Samples were then preserved with trace metal grade nitric acid (trace metal grade, Fisher Scientific, Fair Lawn, NJ, USA) to pH < 2 and refrigerated (0 - 4 °C) prior to analysis.

2.11 ANALYTICAL CONFIRMATION

Samples were analyzed for total and dissolved Ni at the OSU W.M. Keck Collaboratory for Plasma Spectrometry (Corvallis, OR, USA) using a Thermo Scientific X-series II Inductively Coupled Plasma-Mass spectrometer (ICP-MS). Samples were analyzed according to USEPA Method 200.8 (USEPA 1994b). Method blanks were run with each analysis and consisted of deionized water treated identically as the samples through the entire process including acidification. Quality control samples were run in all tests with a standard concentration and an over-spike of a known addition of Ni and analyzed to calculate % recovery for the samples.

2.12 STATISTICAL ANALYSIS

Differences in survival and reproduction were evaluated using a statistical computer package (Comprehensive Environmental Toxicity Information System [CETIS], version 1.8.4.7, Tidepool Scientific Software, version 1.30, McKinleyville, CA, USA) following the USEPA statistical decision tree (USEPA 2002). If the data met the assumptions of normality and homogeneity, the NOEC and LOEC were estimated using an analysis of variance to compare ($p = 0.05$) survival and reproduction (neonates per original female) in the experimental treatments with that observed in the dilution water control.

For the determination of effect concentrations (EC_x), the statistical program, Toxicity Relationship Analysis Program [TRAP] (Duluth, MN, USA) was used for the determination of effect concentrations to reduce survival or reproduction by 10%, 20% and 50% relative to control performance ($LC_{10}/LC_{20}/LC_{50}$ and $EC_{10}/EC_{20}/EC_{50}$). Effect concentrations were estimated using threshold sigmoid regression analysis. Exposure concentrations were log-transformed before determination of the EC_x values and EC_x statistical analyses were conducted using a weighted regression analysis (mean survival or reproduction weighted by standard deviation).

3. RESULTS AND CONCLUSIONS

Records of biological and chemical data collected during testing, and the statistical analyses used for reporting are included in Appendix B (water quality chemistry), C (analytical metals chemistry), and D (raw data and statistical analysis) of this report.

The studies were initially performed by splitting one simulated effluent into 2 aliquots (one had 14 mg/L nominal DOC added while the other received no additional DOC). These tests were conducted concurrently under the same conditions with organisms from the same acclimated culture. The test with DOC achieved control acceptability criteria ($\geq$ 80 % survival and $\geq$ 60% surviving females having 15 or more neonates); however, the test without DOC did not achieve the required reproductive criteria (even as it was allowed to run for 8 days to achieve 3 broods). Control survival criteria (100%) was met, but only 4 replicates (out of 10 replicates) achieved 3 broods. In this initial "without DOC" test, typical reproduction was observed in the 1st and 2nd broods (Days 4 and 5), but no 3rd brood occurred over a total of 8 days. This was a confounding finding, as the control water "with DOC" had similar reproduction (similar to that observed in the controls without DOC) on Days 4 and 5 and also achieved 3 broods within the 7-day test. It is believed that this finding was not due to technician or procedural error, but was possibly due to a change in water chemistry or exposure conditions. One observation during this initial test "without DOC" was precipitation of salts on both the bottom of the carboy holding the water and within the test chamber. This observation was not apparent in the simulated effluent with DOC. In order to achieve control acceptability criteria, the simulated effluent test without DOC was reconducted successfully (and is reported herein). The test with DOC was not repeated and the original test is reported.

3.1 TEST CONDITIONS

Water quality characteristics and measurements for the dilution waters of each test are reported in Tables 3-1 and 3-2, in addition to the water quality parameters measured in the previous experiment (OSU 2016). Due to the use of two separate batches of simulated effluent (discussed in above paragraph), there was some variation in water quality measurements between the two control/dilution waters of the respective tests. Water quality chemistries were measured in both "new" waters that were representative of freshly-prepared solutions after the 3-hr equilibrium period, but before organism exposure, and measured in "old" waters that were a composite of replicate waters following 24-hrs of organism exposure. The control water in the simulated effluent with added DOC test was slightly harder than the test without DOC. Within test variability in the hardness and alkalinity measurements was relatively consistent between the "new" and "old" chemistries. As the toxicity tests were conducted under ambient conditions and were not pH controlled, pH measurements between the "new" waters and "old" waters increased. The increase in pH was more pronounced in the without DOC test, increasing by up to 0.5 SU, whereas the "with DOC" test only increased by 0.1 SU. Acceptable temperatures (25 ± 1°C) and acceptable dissolved oxygen concentrations (saturation $\geq$ 60%) were maintained in both tests.

In order to determine DOC concentrations attributable to food addition (Section 2.9) in the toxicity tests, a series of additional DOC measurements were sampled both in "new" and "old" waters. As demonstrated in Table 3-3, DOC concentrations increased by approximately 1.0 mg/L in the simulated effluent "without DOC" test and increased by approximately 2.0 mg/L in the simulated effluent "with DOC" test. As the addition of food does not immediately translate to an increase of DOC within the test chamber, but could possibly be observed over a course of time, BLM predictions were conducted with multiple DOC input values. One prediction was made with the average of DOC measurements in the "new" waters, one prediction with the average of all measurements, and one prediction with the average of "old" waters.

Table 3-1. Summary of Water Quality Parameters – Toxicity Testing

Test Series	**Test #**	**Nominal Ni Conc. (µg/L)**	**Parameter**									
			Hardness Range (new waters)	Alkalinity Range (new waters)	Hardness Range (old waters)	Alkalinity Range (old waters)	Conductivity Range (new waters)	Conductivity Range (old waters)	TDS Range (new waters)	TDS Range (old waters)	pH Range (new waters)	pH Range (old waters)
			(mg/L as $CaCO_3$)				(µS/cm)		mg/L		(SU)	(SU)
Simulated Effluent **without** DOC	Ni WER 1132R CDC	0 (Control)	248 - 328	328 - 432	248 - 284	352 - 388	2230 - 2350	2270 - 2550	1125 - 1185	1149 - 1293	8.16 - 8.55	8.65 - 8.89
		2.1	-	-	-	-	2270 - 2360	2290 - 3040	1151 - 1194	1158 - 1556	8.18 - 8.57	8.69 - 8.88
		2.9	-	-	-	-	2250 - 2350	2280 - 2560	1138 - 1190	1152 - 1300	8.19 - 8.56	8.69 - 8.89
		4.2	270 - 272	356 – 372	260 - 284	376 - 388	2250 - 2350	2300 - 2570	1139 - 1190	1165 - 1305	8.19 - 8.57	8.68 - 8.89
		6.0	-	-	-	-	2260 - 2350	2280 - 2640	1141 - 1189	1156 - 1339	8.16 - 8.56	8.70 - 8.90
		8.5	-	-	-	-	2250 - 2350	2300 - 2580	1139 - 1189	1160 - 1310	8.17 - 8.57	8.71 - 8.93
		12.2	-	-	-	-	2260 - 2350	2270 - 2590	1137 - 1187	1147 - 1316	8.18 - 8.55	8.68 - 8.89
		17.4	248 - 276	332 - 380	272 - 288	376 - 388	2250 - 2350	2280 - 2830	1137 - 1186	1150 - 1444	8.18 - 8.57	8.69 - 8.90
		VHW RW	284 - 328	180 - 232	288 - 320	184 – 204	928 - 987	976 - 1281	454 - 484	475 - 633	8.41 - 8.66	8.51 - 8.75
Simulated Effluent **with** DOC	Ni WER 1126 CDC	0 (Control)	304 - 325	392 - 408	316 - 316	412 – 420	2270 - 2340	2370 - 2570	1145 - 1184	1198 - 1308	8.54 - 8.80	8.64 - 8.81
		4.5	-	-	-	-	2270 - 2340	2410 - 2840	1148 - 1183	1217 - 1448	8.55 - 8.80	8.65 - 8.80
		6.5	-	-	-	-	2270 - 2340	2390 - 2560	1147 - 1183	1211 - 1302	8.56 - 8.80	8.66 - 8.82
		9.2	304 - 312	396 - 404	316 - 328	416 – 420	2270 - 2340	2430 - 2730	1148 - 1183	1231 - 1392	8.56 - 8.81	8.67 - 8.83
		13.2	-	-	-	-	2270 - 2340	2380 - 2570	1147 - 1182	1203 - 1306	8.56 - 8.80	8.67 - 8.82
		18.9	-	-	-	-	2270 - 2340	2360 - 2590	1147 - 1182	1193 - 1314	8.56 - 8.80	8.68 - 8.81
		26.9	-	-	-	-	2270 - 2340	2410 - 2650	1147 - 1182	1220 - 1349	8.56 - 8.81	8.67 - 8.83
		38.5	304 - 304	396 - 400	328 - 330	420 - 424	2270 - 2340	2370 - 2610	1147 - 1182	1198 - 1326	8.56 - 8.81	8.69 - 8.81

SU = Standard Units.

Table 3-2. Summary of Water Quality Parameters – Control/dilution water

Parameter	Simulated Effluent without DOC (Ni WER 1132R CDC)	Simulated Effluent with DOC (Ni WER 1126 CDC)	Previous Study Simulated Effluent without DOC (OSU 2016)
Calcium (mg/L) :	36.4	46.0	52.5
Magnesium (mg/L) :	46.9	45.8	45.9
Sodium (mg/L) :	379.0	393.0	348.0
Potassium (mg/L) :	86.0	81.9	81.3
Chloride (mg/L) :	348	349	315
Sulfate (mg/L) :	316	321	295

Table 3-3. Water Quality – Dissolved Organic Carbon

Parameter	Simulated Effluent without DOC (Ni WER 1132R CDC)						
	0 (Control)	12.2 µg/L Ni	17.4 µg/L Ni	0 (Control)	12.2 µg/L Ni	17.4 µg/L Ni	Average ± SD
	new			old			
DOC (mg/L) :	< 0.20 [1]	Not sampled	< 0.20 [1]	0.93	Not sampled	1.02	0.54 ± 0.51 *

	Simulated Effluent with DOC (Ni WER 1126 CDC)						
Parameter	0 (Control)	26.9 µg/L Ni	38.5 µg/L Ni	0 (Control)	26.9 µg/L Ni	38.5 µg/L Ni	Average ± SD
	new			old			
DOC (mg/L) :	11.2	11.3	11.4	Not sampled	14.0	13.1	12.20 ± 1.27

[1] Below method detection limit of 0.20 mg/L.

* For the determination of the average value, measured values below the detection limit assigned a value of half the detection limit (0.10 mg/L).

3.2 DEFINITIVE TEST CONCENTRATIONS

Analytical chemistry data is provided in Appendix C. Measured total and dissolved (0.45 µm) Ni in the test without DOC test is reported in Tables 3-4 and 3-5. Measured total and dissolved Ni in the test with added DOC is reported in Tables 3-6 and 3-7. In the Ni spiked concentrations in the test without DOC test, total Ni was 82 - 143% of nominal Ni and dissolved Ni concentrations ranged from 80 - 154% of nominal and 81 - 108% of total Ni. In the Ni spiked concentrations in the test with added DOC test, total Ni was 83 - 133% of nominal Ni and dissolved Ni concentrations ranged from 80 - 129% of nominal and 88 - 114% of total Ni. Although certain measurements were greater than 100%, these differences equate to approximately 1 µg/L or less.

Nickel exposure concentrations are reported under two categories: “new” and “old”. “New” waters were sampled directly from newly prepared waters (following the equilibrium period) prior to test initiation or daily water renewal. “Old” waters consisted of a composite of each replicate directly from the test chamber (representing 24-hrs of exposure). Background Ni concentrations were measured in both control waters, with an average measurement of 1.3 µg/L total Ni in the “without DOC” test and 1.6 µg/L total Ni in the “with DOC” test. Method blanks were run with each analysis and consisted of deionized water treated identically as the samples through the entire process including acidification. All blank measurements from the total recoverable samples were below detection limits, with one exception (0.05 µg/L Ni [DL = 0.023], while method blanks from the dissolved samples measured between 0.04 and 0.18, demonstrating the syringe filter contributed some amount of Ni to the blank samples. Quality control samples were run in all tests with a standard concentration and an over-spike of a known addition of metal and analyzed to calculate % recovery for the samples. Quality control standards of 10 µg/L Ni ranged from 101 - 113% recovery during the analytical run of the simulated effluent without DOC and ranged from 107 - 115% recovery during the analytical run of the simulated effluent with DOC. Standard additions of 9 µg/L Ni ranged from 94 - 106% recovery during the analytical run of the simulated effluent without DOC and ranged from 89 - 101% recovery during the analytical run of the simulated effluent with DOC.

Table 3-4. Summary of Metal Analyses – Simulated Effluent without DOC – Total - (µg/L Ni)

Nominal Conc.	**Measured Total Concentration**					**New waters**		**Old waters**		**All Total**	
	Day 0 new	Day 3 new	Day 4 old	Day 6 new	Day 7 old	**Average Total Measured Conc.**	**Std Dev Total Measured Conc.**	**Average Total Measured Conc.**	**Std Dev Total Measured Conc.**	**Average Total Measured Conc.**	**Std Dev Total Measured Conc.**
VHW RW[1]	1.2	1.1	1.0	Sample error	1.1	1.1	0.1	1.1	0.1	1.1	0.1
0 (Control)	1.3	1.3	1.4	1.5	1.4	1.3	0.1	1.4	0.0	1.4	0.1
2.1	2.9	3.0	2.8	3.0	2.9	3.0	0.1	2.9	0.1	2.9	0.1
2.9	3.5	3.7	4.0	3.7	3.7	3.6	0.1	3.8	0.2	3.7	0.2
4.2	4.5	4.7	4.4	4.7	4.4	4.6	0.2	4.4	0.0	4.5	0.2
6.0	5.9	6.4	5.8	6.5	6.1	6.3	0.3	6.0	0.2	6.1	0.3
8.5	7.9	8.4	8.1	8.3	8.1	8.1	0.3	8.1	0.0	8.1	0.2
12.2	10.5	11.4	10.6	11.4	10.8	11.1	0.5	10.7	0.1	10.9	0.4
17.4	14.4	15.5	14.9	16.3	15.5	15.4	1.0	15.2	0.4	15.3	0.7

[1] Very-hard reconstituted lab water concurrent control exposure used for comparison to simulated effluent with no DOC control/dilution water only. Not used in comparison analysis to Ni exposures.

Table 3-5. Summary of Metal Analyses – Simulated Effluent without DOC – Dissolved - (µg/L Ni)

Nominal Conc.	**Measured Dissolved Concentration**					**New waters**		**Old waters**		**All Dissolved**	
	Day 0 new	Day 3 new	Day 4 old	Day 6 new	Day 7 old	**Average Dissolved Measured Conc.**	**Std Dev Dissolved Measured Conc.**	**Average Dissolved Measured Conc.**	**Std Dev Dissolved Measured Conc.**	**Average Dissolved Measured Conc.**	**Std Dev Dissolved Measured Conc.**
VHW RW[1]	1.2	1.2	1.1	1.3	1.1	1.2	0.1	1.1	0.0	1.2	0.1
0 (Control)	1.3	1.3	1.2	1.5	1.2	1.3	0.1	1.2	0.0	1.3	0.1
2.1	2.9	3.0	2.6	3.2	2.8	3.0	0.2	2.7	0.1	2.9	0.2
2.9	3.5	3.7	3.2	3.9	3.3	3.7	0.2	3.3	0.1	3.5	0.3
4.2	4.5	4.8	4.1	4.9	4.0	4.7	0.2	4.1	0.1	4.5	0.4
6.0	5.9	6.5	5.6	6.7	5.5	6.4	0.4	5.5	0.1	6.0	0.5
8.5	7.9	8.5	7.5	8.6	7.3	8.3	0.4	7.4	0.1	8.0	0.6
12.2	10.6	11.5	10.1	11.7	10.1	11.3	0.6	10.1	0.0	10.8	0.8
17.4	14.6	16.0	14.0	16.4	14.7	15.7	0.9	14.3	0.5	15.1	1.0

[1] Very-hard reconstituted lab water concurrent control exposure used for comparison to simulated effluent with no DOC control/dilution water only. Not used in comparison analysis to Ni exposures.

Table 3-6. Summary of Metal Analyses – Simulated Effluent with added DOC – Total - (µg/L Ni)

Nominal Conc.	**Measured Total Concentration**					**New waters**		**Old waters**		**All Total**	
	Day 0 new	Day 3 new	Day 4 old	Day 6 new	Day 7 old	**Average Total Measured Conc.**	**Std Dev Total Measured Conc.**	**Average Total Measured Conc.**	**Std Dev Total Measured Conc.**	**Average Total Measured Conc.**	**Std Dev Total Measured Conc.**
0 (Control)	1.6	1.5	1.5	1.6	1.5	1.6	0.1	1.5	0.0	1.5	0.1
4.5	6.0	4.9	5.0	5.1	5.3	5.3	0.6	5.2	0.2	5.3	0.4
6.5	6.4	6.4	6.6	6.6	7.3	6.5	0.1	7.0	0.5	6.7	0.4
9.2	8.5	8.6	8.5	10.4	8.8	9.2	1.1	8.7	0.2	9.0	0.8
13.2	12.2	11.8	11.8	12.2	11.9	12.1	0.2	11.9	0.1	12.0	0.2
18.9	16.2	16.3	17.1	17.8	17.6	16.8	0.9	17.4	0.4	17.0	0.7
26.9	22.2	22.7	23.6	23.5	23.2	22.8	0.7	23.4	0.3	23.0	0.6
38.5	32.6	31.9	32.1	34.6	32.0	33.0	1.4	32.1	0.1	32.6	1.1

Table 3-7. Summary of Metal Analyses – Simulated Effluent with added DOC – Dissolved - (µg/L Ni)

Nominal Conc.	Measured Dissolved Concentration					New waters		Old waters		All Dissolved	
	Day 0 new	Day 3 new	Day 4 old	Day 6 new	Day 7 old	Average Dissolved Measured Conc.	Std Dev Dissolved Measured Conc.	Average Dissolved Measured Conc.	Std Dev Dissolved Measured Conc.	Average Dissolved Measured Conc.	Std Dev Dissolved Measured Conc.
0 (Control)	1.6	1.6	1.6	1.7	1.5	1.6	0.1	1.6	0.1	1.6	0.1
4.5	5.7	5.2	5.1	5.8	5.0	5.6	0.3	5.1	0.1	5.4	0.4
6.5	7.0	6.8	6.8	7.0	6.5	6.9	0.1	6.7	0.2	6.8	0.2
9.2	9.2	9.1	8.9	9.2	8.4	9.2	0.1	8.7	0.4	9.0	0.3
13.2	13.3	12.3	12.2	12.1	11.5	12.6	0.6	11.9	0.5	12.3	0.6
18.9	17.4	17.0	17.5	18.0	17.0	17.5	0.5	17.3	0.4	17.4	0.4
26.9	24.6	23.7	23.8	23.2	23.0	23.8	0.7	23.4	0.6	23.7	0.6
38.5	33.3	33.1	32.5	32.9	30.9	33.1	0.2	31.7	1.1	32.5	1.0

3.3 BIOLOGICAL RESULTS

A copy of the raw biological data (including statistical print-outs) is provided in Appendix D. Summary tables for each study are presented in Tables 3-8 through 3-9. In both tests reported here, control acceptability criteria (≥ 80 % survival and ≥ 60% surviving females having 15 or more neonates) were met. There was also no statistically significant difference between the simulated effluent "without DOC" and the concurrent very hard reconstituted water.

To determine effect concentrations in each test, survival and reproduction in the Ni exposures (based upon average measured dissolved Ni) were compared to their respective dilution water control in each test. The exposures where a survival effect was identified (LOEC of 10.8 µg/L dissolved Ni and above in the simulated effluent "without DOC" test and a LOEC of 32.5 µg/L dissolved Ni in the "with DOC" test) were not used in the NOEC/LOEC determinations for reproduction, resulting in reproductive LOEC of > 8.0 µg/L dissolved Ni in the "without DOC" test and a LOEC of 17.4 µg/L dissolved Ni in the "with DOC" test. Based upon % effect concentrations (EC_x), reproduction was more sensitive than survival and the simulated effluent "with DOC" test was less sensitive than its no-DOC counterpart. A summary of the statistical endpoints for the tests are presented in Table 3-10.

Table 3-8. Summary of Biological – Simulated Effluent without DOC

Average Dissolved Measured Conc. (µg/L Ni)	Survival (Proportion Survived)		Reproduction (young per original female)	
	Average	Std Dev	Average	Std Dev
1.2 (VHW RW)[1]	1.00	0	31.7	6.5
1.3 (Control)	1.00	0	26.9	6.8
2.9	1.00	0	28.1	5.6
3.5	1.00	0	29.2	5.5
4.5	0.90	0.32	27.1	9.0
6.0	1.00	0	28.6	2.2
8.0	0.80	0.42	17.2	10.9
10.8	0.50 *	0.53	12.0 **	6.5
15.1	0.20 *	0.42	9.2 **	6.0

[1] Very-hard reconstituted lab water concurrent control exposure used for comparison to simulated effluent with no DOC control/dilution water only. Not used in comparison analysis to Ni exposures. Exposure not statistically different from simulated effluent without DOC control/dilution water.

* Significantly less than control (p=0.05) using Dunnett Multiple Comparison Test or Steel Many-One Rank Sum Test.

** Exposure concentrations which exhibit an effect on survival are not included in the determination of NOEC/LOEC for reproduction, but are included in ECx calculations.

Table 3-9. Summary of Biological – Simulated Effluent with added DOC

Average Dissolved Measured Conc. (µg/L Ni)	Survival (Proportion Survived)		Reproduction (young per original female)	
	Average	Std Dev	Average	Std Dev
1.6 (Control)	1.00	0	39.4	4.9
5.4	0.90	0.32	37.1	13.2
6.8	0.90	0.32	37.7	10.1
9.0	1.00	0	37.5	2.3
12.3	1.00	0	35.7	3.7
17.4	1.00	0	28.5 *	8.7
23.7	0.90	0.32	19.0 *	7.7
32.5	0.33 *	0.50	10.1 **	7.6

* Significantly less than control (p=0.05) using Fisher Exact/Bonferroni-Holm Test or Steel Many-One Rank Sum Test.

** Exposure concentrations which exhibit an effect on survival are not included in the determination of NOEC/LOEC for reproduction, but are included in ECx calculations.

Table 3-10. Summary of Statistics

Test Description	**Survival**					**Reproduction (young per original female)**				
	NOEC	**LOEC**	**LC_{10} (95% CI)**	**LC_{20} (95% CI)**	**LC_{50} (95% CI)**	**NOEC**	**LOEC**	**EC_{10} (95% CI)**	**EC_{20} (95% CI)**	**EC_{50} (95% CI)**
	µg/L dissolved Ni									
Simulated Effluent without DOC Ni WER 1132R CDC	8.0	10.8	7.1 (6.0 - 8.5)	8.3 (7.3 - 9.4)	11.0 (10.2 - 12.0)	8.0 *	> 8.0 *	6.8 (4.8 - 9.8)	8.0 (6.1 - 10.6)	11.0 (9.0 - 13.5)
Simulated Effluent with DOC Ni WER 1126 CDC	23.7	32.5	24.4 (21.2 -28.2)	26.3 (23.6 - 29.3)	30.4 (28.5 - 32.4)	12.3 *	17.4 *	13.2 (11.6 - 15.0)	16.1 (14.6 - 17.7)	24.0 (22.5 - 25.6)

NOEC = No observable effect concentration, LOEC = Lowest observable effect concentration, LC_x = 10%/20%/50% lethal effect concentrations. EC_x = 10%/20%/50% reproductive effect concentrations.

95% CI = 95% confidence intervals.

* Exposure concentrations with a significant effect on survival not included in the NOEC/LOEC determination for reproduction.

3.4 CONCLUSION

Based upon the water quality characteristics reported in Tables 3-2 and 3-3, the Nickel Biotic Ligand Model (BLM) (HDR 2013) was used to assess differences between model predicted effects versus actual observed effects (Table 3-11). In the simulated effluent water without added DOC, the BLM predicted (using a range of DOC concentrations from 0.10 to 0.98 mg/L, as explained in Section 3.1) a LC_{20} of 8.7 – 11.5 and an EC_{20} of 4.8 – 6.4 µg/L Ni for survival and reproduction, respectively. This is compared with the observed effect of a survival LC_{20} of 8.3 µg/L dissolved Ni and a reproductive EC_{20} of 8.0 µg/L dissolved Ni. In addition to the present study, the previous study (OSU 2016) of simulated effluent without added DOC (with slight variations in measured water quality) resulted in a reproductive EC_{20} of 7.4 µg/L dissolved Ni which was very similar to the reproductive EC_{20} achieved in this study (i.e., EC_{20} of 8.0 µg/L dissolved Ni). In the simulated effluent with the addition of DOC, the BLM predicted higher than observed toxic concentrations (i.e., BLM predicted less than observed toxicity), predicting a LC_{20} of 60.9 – 71.2 and an EC_{20} of 34.8 – 40.8 µg/L dissolved Ni compared to the observed LC_{20} of 26.3 and EC_{20} of 16.1 µg/L dissolved Ni. Because certain water quality parameters (in terms of hardness, alkalinity, and pH) varied between the "without added DOC" and "with added DOC" tests, these two tests cannot be equally compared based upon DOC alone. Overall, the BLM preformed reasonably well, accurately predicting Ni toxicity in low DOC waters (up to 0.98 mg/L DOC). However, the BLM did not perform as well predicting Ni toxicity in waters containing > 1.0 mg/L DOC. It should be noted that because the BLM does not provide confidence intervals with its predictions, it is difficult to assess whether there were statistically significant differences between observed and predicted outcomes.

Table 3-11. Nickel Biotic Ligand Model (BLM) Predicted versus Observed

Test water	Survival			Reproduction		
	NOEC	LOEC	LC_{20} (95% CI)	NOEC	LOEC	EC_{20} (95% CI)
	Present study					
Simulated effluent **without** added DOC	8.0	10.8	8.3 (7.3 – 9.4)	8.0	> 8.0	8.0 (6.1 – 10.6)
BLM prediction ("new" water 0.10 mg/L)	-	-	8.7	-	-	4.8
BLM prediction (average DOC value 0.54 mg/L)	-	-	10.1	-	-	5.6
BLM prediction ("old" water DOC 0.98 mg/L)	-	-	11.5	-	-	6.4
Simulated effluent **with** added DOC	23.7	32.5	26.3 (23.6 – 29.3)	12.3	17.4	16.1 (14.6 – 17.7)
BLM prediction ("new" water 11.3 mg/L)	-	-	60.9	-	-	34.8
BLM prediction (average DOC value 12.2 mg/L)	-	-	64.9	-	-	37.2
BLM prediction ("old" water DOC 13.6 mg/L)	-	-	71.2	-	-	40.8
	Previous study					
Simulated effluent **without** added DOC	12.6	18.2	13.0 (11.8 – 14.3)	4.7	6.4	7.4 (5.2 – 10.5)
BLM prediction	-	-	13.77	-	-	7.24

3.5 PROTOCOL DEVIATIONS AND AMENDMENTS

During the course of the studies two separate simulated effluents were prepared, one for each test. This occurred due to the inability of the first study “without DOC” to achieve control acceptability criteria (60% of surviving females did not achieve a 3^{rd} brood by 8 days). It is unknown why the test did not achieve acceptability as the same batch of test organisms was used in the concurrent “with DOC” test and acceptability was achieved in that test. It was noted that salts precipitated out of solution in the “without DOC” test, but this was not observed in the “with DOC” test. Due to this protocol deviation, a complete side by side comparison based solely upon DOC is unable to occur. As one of the goals for this study was to determine the accuracy of the BLM to predict observed values, measured water quality parameters allow this prediction to occur.

3.6 LOCATION OF RAW DATA ARCHIVES

The raw data and final report for this study are archived in the OSU AquaTox archives.

4. REFERENCES

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APPENDIX A

Protocol

OSU Aquatic Toxicology Laboratory
33972 Texas St. SW
Albany, Oregon 97321 USA
Office 541-737-2565
Lab 541-926-1254

Title:	Short-term Chronic Nickel Toxicity in Simulated Effluent Laboratory Water with and without added Dissolved Organic Carbon (DOC) to the Cladoceran, *Ceriodaphnia dubia*, under Static-Renewal Test Conditions
Testing Facility:	Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox) 33972 Texas Street SW Albany, OR 97321 USA
Study Sponsor:	Sanitary District of Decatur 501 S. Dipper Lane Decatur, Illinois 62522
Sponsor's Study Officer:	Timothy R. Kluge, Project Advisor
Principal Investigator:	William Stubblefield, Ph.D.
Study Director:	Allison Cardwell

1.0 INTRODUCTION

1.1 Objective

To determine the short-term chronic effects of nickel (Ni) on the freshwater cladoceran, *Ceriodaphnia dubia*, in a simulated effluent laboratory reconstituted water with and without added dissolved organic carbon (DOC), under static-renewal test conditions. Results from these toxicity tests will provide empirical data to be used as a validation exercise of the Ni biotic ligand model (BLM).

1.2 Experimental Approach

Two separate tests will be conducted exposing *C. dubia* to differing concentrations of nickel in a simulated effluent laboratory reconstituted waters with and without added DOC during sub-chronic aqueous exposures. DOC will be added at a concentration measured in site effluent from the Sanitary District of Decatur (SDD; Decatur, Illinois).

1.3 Test Substance

The test substance will be in the form of reagent-grade nickel chloride hexahydrate ($NiCl_2$ x $6H_2O$; CAS # 7791-20-0).

2.0 BASIS AND TEST SYSTEM

2.1 Basis

This protocol is designed to comply with USEPA testing guidance (USEPA 2002).

2.2 Test Species

1. Species: Cladoceran/Water Flea (*Ceriodaphnia dubia*).
2. Number: Each test will consist of 10 replicates for each treatment and control(s).
3. *C. dubia* will start as less than 24 hr old neonates.
4. Source: *C. dubia* are cultured at Oregon State University's Aquatic Toxicology Lab (OSU AquaTox, Albany, OR).
5. Culture/Holding Water: For acclimation of organisms to the expected ionic makeup of the SDD site effluent water, *C. dubia* adults are maintained individually in 30 mL plastic containers in diluted reconstituted laboratory water. The reconstituted water is prepared to simulate the SDD site effluent (simulated based upon Ca, Mg, Na, K, SO_4, Cl) and diluted by 20% prior to use as a culture medium. Following the dilution, DOC will be added in the form of Suwannee River Isolate (International Humic Substances Society [IHSS]). This culture medium is prepared as described in Section 3.2. Survival and reproduction of the test organisms is monitored daily to ensure acceptable organism health (assessed by laboratory personnel). Survival and health of the organisms must be acceptable for at least two weeks prior to testing, organisms will be maintained individually in 30 mL plastic containers in an environmental chamber.
6. Feeding: Each chamber will be fed 0.3 ml of a Yeast/Trout Chow/Cereal leaves mixture (YTC) and algae suspension (*Pseudokirchneriella subcapitata*, 1:1), daily during renewal.

7. Procedure for identification: *C. dubia* have been verified to species by the original organism supplier.

2.3 Test Diet

The diet used is composed of an YTC and *Pseudokirchneriella subcapitata* suspension as outlined in OSU AquaTox Standard Operating Procedure (SOP) 5107.

3.0 EXPOSURE SYSTEM

3.1 Route of Administration

Equipment: A concentrated stock solution of the test substance will be prepared with a reagent grade salt (see section 1.3) and will be weighed/apportioned using an electronic micro-balance. The stock solution will be added to test waters using a micro-pipette.

Method: Appropriate volumes of nickel stock will be added individually to each dilution water (with and without DOC) (see Section 3.2) to achieve nominal concentrations. Following the spiking of nickel to each concentration, the waters will equilibrate for 1-3 hours prior to use.

Frequency: A 100% renewal of control and treatment solutions will occur daily by transferring each adult organism to a freshly prepared exposure chamber. Each day, solutions will equilibrate for 1-3 hours prior to use. The equilibration period will be the same (1-hr, 2-hr, or 3-hr) on each day of renewal.

3.2 Dilution Water

Simulated Effluent/Laboratory Water
Dilutions water for the tests will be diluted simulated effluent reconstituted water (with and without DOC). The water will be prepared as follows:

- Addition of the appropriate reagent grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, NaCl, and $NaHCO_3$) to deionized water achieve a nominal hardness and alkalinity of approximately 400 mg/L as $CaCO_3$ and 400 mg/L as $CaCO_3$.
- Bubble solution with CO_2 to reduce pH and promote carbonate dissolution. Reduce pH to below 6.0. Leave water in a zero headspace environment overnight.
- Bubble solution with O_2 to increase pH to 8.2 - 8.5.
- Dilute water by 20% with deionized water.
- Aerate water overnight.
- Split the water in half: one portion to be used in the test without DOC and one portion to have added DOC and to be used in the test with DOC.
- For the “with DOC” test only: Add Suwannee River Natural Organic Matter Isolate (NOM; obtained from International Humic Substances Society) to achieve a nominal DOC concentration of 14 mg/L (based on a composition of 48% DOC in the NOM). Aerate overnight before use.
- Characterize hardness, alkalinity, pH, and conductivity of final dilution waters prior to use as control/dilution for the toxicity test.

There will be one concurrent exposure, consisting of USEPA very hard water (USEPA 2002), included in the study.

3.3 Test Temperature

Test temperature will be 25 ± 2 °C. Testing will be conducted in a temperature-controlled environmental chamber.

3.4 Test Chamber

Test containers will be 30-mL plastic Soufflé cups containing 25-mL of test solution. Containers will be covered with Plexiglas to prevent contamination.

3.5 Photoperiod

Lighting for the entire test duration will be a photoperiod of 16-hours light and 8-hours dark, provided by cool-white or daylight illumination.

3.6 Dissolved Oxygen Concentrations

Dissolved oxygen concentrations will be maintained at ≥ 60 percent of saturation.

4.0 TEST DESIGN

4.1 Test Concentrations/Dosages

For each test, seven Ni treatments and a dilution water control will be tested using a 0.7 dilution scheme. The nominal test concentrations will be estimated based upon range finding experiments and historical data. Nominal test concentrations will be described in the raw data packet. One concurrent treatment of very hard reconstituted control water (USEPA 2002; without nickel) will also be tested.

4.2 Number of Test Organisms

Each test will consist of ten replicates for each treatment and control. One *C. dubia* neonate will be partitioned into each test vessel at the start of the test.

4.3 Bias Control

To control bias, test chambers will be numbered according to a 10 X 8 randomization sheet (for each test) and placed in the environmental chamber.

4.4 Test Initiation

After collection, the neonates from a single adult will be distributed into one row of randomly ordered test chambers, with only one neonate transferred into each test chamber. This process will be repeated using a new brood of neonates from a single adult for each row of the entire randomization pattern to initiate testing.

4.5 Chemical and Physical Monitoring

At a minimum, the following measurements will be made according to the methods laid out in OSU AquaTox SOPs:

1. Hardness, alkalinity, dissolved oxygen, temperature, conductivity, total ammonia, total residual chlorine, total dissolved solids, and pH will be measured in the simulated effluent/laboratory water at test initiation. Hardness and alkalinity of the control(s), one middle concentration and the highest concentration, will also be measured at the Day 3 and 6 renewal time point (of both new renewal waters and old waters) and at test termination.

2. A sample of the simulated effluent/laboratory control water and a sample of the highest Ni exposure will be collected for characterization of calcium, magnesium, sodium, potassium, chloride, sulfate, and dissolved organic carbon (DOC) and measured at an outside commercial laboratory. These samples will be collected from "new" waters, following the equilibrium period, but prior to use for test initiation or water renewal.

3. Additional samples for DOC analysis will be collected from "old waters" from samples taken after 24 hours of exposure. "Old" waters will be a composite sample of each replicate of a treatment after the transfer of the original organism. One "old" samples will be taken from the simulated effluent/laboratory water control and one "old" sample will be taken from the highest Ni exposure.

4. Dissolved oxygen, temperature, conductivity, and pH will be measured daily in each treatment (of both new renewal waters and old waters).

4.6 Biological Monitoring

Observations of live and dead organisms, as well as neonates produced, will be recorded daily. Only adult females will be transferred daily to fresh solutions.

4.7 Analytical Chemistry

Samples for nickel analysis will be collected from each treatment according to the following schedule: On Day 0 (initiation), samples for total recoverable (unfiltered and acidified with concentrated nitric acid to a pH < 2) and dissolved (filtered through 0.45 µm-porosity filter prior to acidification) will be collected separately into a 15 ml polypropylene conical tube from each treatment. Samples for analysis of total and dissolved nickel will also be collected from new renewal waters on Day 3 and 6 and from old test waters (from a composite of the ten replicates for each treatment) on Day 4 and at test termination. Filters (0.45 µm-porosity) used for dissolved metal collections will be flushed with 5 ml of sample prior to sample collection. Total recoverable and dissolved nickel samples will be analyzed via Inductively Coupled Plasma Optical Emission Spectrometry or Mass Spectrometry (ICP-OES/MS) (USEPA 1994a, USEPA 1994b)

Certain water quality parameters will be measured at an outside commercial laboratory, CH2M (Corvallis, OR, USA). Calcium, magnesium, sodium, and potassium will be measured via Inductively Coupled Plasma (ICP) Atomic Emission Spectroscopy (EPA 200.7; USEPA 1994a). Chloride and sulfate will be measured via Ion Chromatography (EPA 300.0; USEPA 1993) and dissolved organic carbon (DOC) will be measured via Combustion (Standard Methods 5310B; APHA 1998).

4.8 Test Duration

The test duration will be 7 days at a minimum, but can go for 8 days if necessary for control organisms (i.e. non-exposed organisms) to produce a third brood.

4.9 Quality Criteria

- Each test will not be considered valid if control mortality (non-spiked) exceeds 20% or if control organisms fail to produce an average of ≥ 15 neonates per surviving female, or if a third brood is not produced by ≥ 60% of surviving control organisms within 8 days.
- The dissolved oxygen concentration must be > 60 percent saturation.
- There must be evidence that the temperature, dissolved oxygen, and concentration of the test substance being tested have been satisfactorily maintained, based on time-weighted averages, over the test period.

5.0 DATA ANALYSIS

For each test, statistical analysis (hypothesis testing) of the test data will be conducted using a computer program, Comprehensive Environmental Toxicity Information System (CETIS). A statistical test (as determined by the USEPA Decision Tree [USEPA, 2002]) will be used to test for significant differences in the survival and reproduction among test treatments and controls. The no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC) will be calculated on the basis of survival and reproduction ($p < 0.05$). In addition, using Toxicity Relationship Analysis Program (TRAP, version 1.30a, Erickson 2015), the median lethal concentration (LC_{50}) and 10% or 20% survival or reproductive inhibition concentration (e.g. EC_{10} and EC_{20}) will be calculated along with the determination of outliers and the need for data transformation (i.e. arc sine, square root, logarithmic, etc.).

6.0 TEST REPORT

The report will be a typed document describing the results of the test and will be signed by the Principal Investigator and Study Director. The report will include, but not be limited to, the following:

- Name and address of the test facility;
- Dates of test initiation, completion, and/or termination;
- Objectives of the study as stated in the test protocol, including any changes from the protocol;
- Statistical methods used in data analysis;
- Identification of the test substances (by name, CAS number, or code number) and description of substance purity, strength, composition, stability, solubility, and/or other appropriate characteristics documented by the Study Sponsor (location of documentation shall be specified);
- A description of the methods used during testing;
- A description of the test system used including, where applicable, source of supply, species, strain, sub-strain, age, and procedure for identification;
- A description of the exposure concentrations, dosing regimen, route of administration, and duration of exposure;

- A description of all circumstances that may have affected the quality and/or integrity of the data;
- The name of the Principal Investigator and Study Director and the names of other scientists, professionals, or supervisory personnel (e.g. task manager, senior biomonitoring technician) involved in the study;
- A description of the methods of data analysis; a summary and analysis of the data, and a statement of the conclusions drawn from the analysis;
- Signature and date of the Study Director and/or other professionals involved in the study as required by the testing facility or Sponsor;
- The location(s) where all specimens, raw data, and final report are to be stored;
- A statement of Quality Assurance

7.0 RECORD RETENTION

All records will be maintained and archived in the OSU AquaTox archives in accordance with OSU AquaTox SOP 5403.

8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments, deviations, and final report revisions) of the approved protocol, plus the reasons for the changes, must be documented in writing. The changes will be signed and dated by the Study Director and maintained with the protocol.

9.0 LITERATURE CITED

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater, 20th edition. Washington, D.C.

United States Environmental Protection Agency (USEPA). 1993. Determination of Inorganic Anions by Ion Chromatography in Methods for the Determination of Inorganic Substances in Environmental Samples, Method 300.0 (EPA/600/R-93/100). U.S. EPA National Exposure Research Laboratory (NERL).

USEPA. 1994a. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry in Methods for the Determination of Metals in Environmental Samples, Supplement 1, Method 200.7 (EPA/600/R-94/111). U.S. EPA National Exposure Research Laboratory (NERL) [formerly EMSL].

USEPA. 1994b. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Mass Spectrometry in Methods for the Determination of Metals in Environmental Samples, Method 200.8 (EPA/600/R-94/111). U.S. EPA National Exposure Research Laboratory (NERL) [formerly EMSL].

USEPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013.

10.0 SPONSOR AND STUDY DIRECTOR APPROVAL

Sponsor:

Print Name: Timothy R. Kluge
Signature: Timothy R Kluge
Title: Project Advisor for SDD
Date: 11/22/2016

Principal Investigator:

Print Name: William Stubblefield
Signature:
Title: Professor
Date: 22 Nov 2016

Study Director:

Print Name: Allison Cardwell
Signature: Allison Cardwell
Title: Senior Faculty Research Assistant
Date: 22 November 2016

Page
OSU AquaTox QA Form No. 007
Revision: 0
Effective: 2/10

SUBJECT: SOP/PROTOCOL DEVIATION LOG

Project and Test Nos.: Nickel Simulated Effluent with and without DOC
Date of Occurrence: 24 December 2016
Recorded by: ASC Protocol or SOP Deviation? Protocol

Description of Deviation:

Section 3.2 Dilution Water
The protocol and test design was written to prepare one simulated effluent which would be split into 2 aliquots, of which one would have DOC added to it (and would be conducted as the "with DOC" test). The studies were initially performed by splitting one simulated effluent into 2 aliquots (one had 14 mg/L nominal DOC added while the other received no additional DOC). These tests were conducted concurrently under the same conditions with organisms from the same acclimated culture. The test with DOC achieved control acceptability criteria (≥ 80 % survival and ≥ 60% surviving females having 15 or more neonates); however, the test without DOC did not achieve the required reproductive criteria (even as it was allowed to run for 8 days to achieve 3 broods). Control survival criteria (100%) was met, but only 4 replicates (out of 10 replicates) achieved 3 broods. In this initial "without DOC" test, typical reproduction was observed in the 1st and 2nd broods (Days 4 and 5), but no 3rd brood occurred over a total of 8 days. This was a confounding finding, as the control water "with DOC" had similar reproduction (similar to that observed in the controls without DOC) on Days 4 and 5 and also achieved 3 broods within the 7-day test. This observation was not apparent in the simulated effluent with DOC. In order to achieve control acceptability criteria, the simulated effluent test without DOC was reconducted successfully. The test with DOC was not repeated and the original test is reported.

Actions Taken: (e.g., amendment issued, SOP revision, none - one time deviation, etc.)

The "without DOC" test was reconducted due to the inability of the controls to achieve acceptability criteria in the original test, but the "with DOC" was not re-conducted (as the original achieved acceptability criteria) and the original test reported.

Impact on the Study:

It is believed that the failure of the original "without DOC" test was not due to a technician or procedural error, but was possibly due to a change in water chemistry or exposure conditions. One observation during this original test "without DOC" was precipitation of salts on both the bottom of the carboy holding the water and within the test chamber. Because both tests were not re-conducted, a "side-by-side" with only a difference in DOC cannot be compared, as some of the water quality parameters were different between tests ("without DOC" had lower hardness, alkalinity, and larger pH range from new to old waters).

As one goal of the study was to compare Ni BLM predictions versus observed effects, this effort can still occur as water quality parameters were measured in all tests.

Study Director Signature (if applicable): Allison Cardwell **Date:** 1/9/2017

APPENDIX B

Water Quality Summaries

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia* , in a simulated effluent (no DOC added)
Test #: Ni WER 1132R CDC
Water Quality Summary

	VHW RW (Concurrent)		DILUTED SIMULATED EFFLUENT/LAB WATER no DOC		4.2		17.4	
	NEW							
TEST DAY	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity
0	328	232	328	432	Day 0 not measured	Day 0 not measured	Day 0 not measured	Day 0 not measured
3	288	184	272	380	272	372	276	380
6	284	180	248	328	270	356	248	332
MIN	**284**	**180**	**248**	**328**	**270**	**356**	**248**	**332**
MAX	**328**	**232**	**328**	**432**	**272**	**372**	**276**	**380**
Average	**300**	**199**	**283**	**380**	**271**	**364**	**262**	**356**
Stdev	**24**	**29**	**41**	**52**	**1**	**11**	**20**	**34**

	VHW RW (Concurrent)		DILUTED SIMULATED EFFLUENT/LAB WATER no DOC		4.2		17.4	
	OLD							
TEST DAY	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity
3	320	204	284	388	284	388	288	388
6	288	184	248	352	260	376	272	376
MIN	**288**	**184**	**248**	**352**	**260**	**376**	**272**	**376**
MAX	**320**	**204**	**284**	**388**	**284**	**388**	**288**	**388**
Average	**304**	**194**	**266**	**370**	**272**	**382**	**280**	**382**
Stdev	**23**	**14**	**25**	**25**	**17**	**8**	**11**	**8**

ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia* , in a simulated effluent with added DOC
Test #: Ni WER 1126 CDC
Water Quality Summary

	VHW RW (Concurrent)		**DILUTED SIMULATED EFFLUENT/LAB WATER with DOC**		**9.2**		**38.5**	
	NEW							
TEST DAY	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity
0	336	228	325	392	Day 0 not measured	Day 0 not measured	Day 0 not measured	Day 0 not measured
3	324	224	304	400	304	404	304	400
6	284	184	304	408	312	396	304	396
MIN	**284**	**184**	**304**	**392**	**304**	**396**	**304**	**396**
MAX	**336**	**228**	**325**	**408**	**312**	**404**	**304**	**400**
Average	**315**	**212**	**311**	**400**	**308**	**400**	**304**	**398**
Stdev	**27**	**24**	**12**	**8**	**6**	**6**	**0**	**3**

	VHW RW (Concurrent)		**DILUTED SIMULATED EFFLUENT/LAB WATER with DOC**		**9.2**		**38.5**	
	OLD							
TEST DAY	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity	Hardness	Alkalinity
3	336	236	316	420	316	420	328	424
6	316	212	316	412	328	416	330	420
MIN	**316**	**212**	**316**	**412**	**316**	**416**	**328**	**420**
MAX	**336**	**236**	**316**	**420**	**328**	**420**	**330**	**424**
Average	**326**	**224**	**316**	**416**	**322**	**418**	**329**	**422**
Stdev	**14**	**17**	**0**	**6**	**8**	**3**	**1**	**3**

ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia* , in a simulated effluent
Test #: Ni WER 1132R CDC
Water Quality Summary

	VHW RW (Concurrent)						DILUTED SIMULATED EFFLUENT/LAB WATER						2.1						2.9					
	NEW						NEW						NEW						NEW					
TEST DAY	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS
0	8.50	3.2E-09	25	8.7	987	484	8.55	2.8E-09	25	8.4	2350	1185	8.57	2.7E-09	25	8.4	2360	1194	8.56	2.8E-09	25	8.4	2350	1190
1	8.66	2.2E-09	25	8.6	979	482	8.49	3.2E-09	25	8.5	2310	1165	8.51	3.1E-09	25	8.4	2330	1176	8.50	3.2E-09	25	8.4	2320	1176
2	8.58	2.6E-09	25	8.7	963	472	8.27	5.4E-09	25	8.5	2300	1161	8.28	5.2E-09	25	8.4	2310	1167	8.28	5.2E-09	25	8.4	2310	1168
3	8.54	2.9E-09	25	8.3	947	464	8.16	6.9E-09	25	8.3	2260	1139	8.18	6.6E-09	25	8.3	2280	1153	8.19	6.5E-09	25	8.2	2280	1152
4	8.60	2.5E-09	25	8.6	928	454	8.29	5.1E-09	25	8.6	2230	1125	8.28	5.2E-09	25	8.6	2270	1151	8.28	5.2E-09	25	8.5	2250	1138
5	8.50	3.2E-09	25	8.9	950	467	8.18	6.6E-09	25	8.8	2290	1157	8.20	6.3E-09	25	8.7	2310	1166	8.22	6E-09	25	8.7	2310	1166
6	8.41	3.9E-09	25	8.8	955	470	8.19	6.5E-09	25	8.7	2320	1168	8.20	6.3E-09	25	8.7	2320	1172	8.21	6.2E-09	25	8.7	2330	1177
MIN	**8.41**		**25**	**8.3**	**928**	**454**	**8.16**		**25**	**8.3**	**2230**	**1125**	**8.18**		**25**	**8.3**	**2270**	**1151**	**8.19**		**25**	**8.2**	**2250**	**1138**
MAX	**8.66**		**25**	**8.9**	**987**	**484**	**8.55**		**25**	**8.8**	**2350**	**1185**	**8.57**		**25**	**8.7**	**2360**	**1194**	**8.56**		**25**	**8.7**	**2350**	**1190**
Average	**8.53**	**3E-09**	**25**	**8.7**	**958**	**470**	**8.28**	**5E-09**	**25**	**8.5**	**2294**	**1157**	**8.29**	**5E-09**	**25**	**8.5**	**2311**	**1168**	**8.30**	**5E-09**	**25**	**8.5**	**2307**	**1167**
Stdev	**0.08**		**0.0**	**0.2**	**20**	**10**	**0.16**		**0.0**	**0.2**	**40**	**20**	**0.16**		**0.0**	**0.2**	**30**	**15**	**0.15**		**0.0**	**0.2**	**33**	**17**

	VHW RW (Concurrent)						DILUTED SIMULATED EFFLUENT/LAB WATER						2.1						2.9					
	OLD						OLD						OLD						OLD					
TEST DAY	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS
1	8.75	1.8E-09	25	9	1028	505	8.76	1.7E-09	25	9.3	2550	1293	8.74	1.8E-09	25	9.1	3040	1556	8.80	1.6E-09	25	9.4	2560	1300
2	8.68	2.1E-09	25	9	1281	633	8.75	1.8E-09	25	9.1	2350	1186	8.74	1.8E-09	25	9	2470	1252	8.77	1.7E-09	25	9.2	2440	1235
3	8.65	2.2E-09	25	9.1	976	475	8.79	1.6E-09	25	9	2280	1154	8.77	1.7E-09	25	8.8	2420	1226	8.79	1.6E-09	25	8.9	2470	1220
4	8.68	2.1E-09	25	9.1	1038	509	8.89	1.3E-09	25	9.2	2270	1149	8.88	1.3E-09	25	9.2	2290	1158	8.89	1.3E-09	25	9.3	2280	1152
5	8.61	2.5E-09	25	9.6	1085	534	8.82	1.5E-09	25	9.9	2360	1191	8.82	1.5E-09	25	9.7	2400	1210	8.86	1.4E-09	25	9.9	2370	1192
6	8.58	2.6E-09	25	9.2	1074	528	8.72	1.9E-09	25	9.4	2350	1188	8.77	1.7E-09	25	9.2	2490	1260	8.80	1.6E-09	25	9.4	2370	1200
7	8.51	3.1E-09	25	9.0	1066	527	8.65	2.2E-09	25	9	2410	1220	8.69	2E-09	25	9.0	2500	1269	8.69	2E-09	25	9.0	2530	1282
MIN	**8.51**		**25**	**9**	**976**	**475**	**8.65**		**25**	**9**	**2270**	**1149**	**8.69**		**25**	**8.8**	**2290**	**1158**	**8.69**		**25**	**8.9**	**2280**	**1152**
MAX	**8.75**		**25**	**9.6**	**1281**	**633**	**8.89**		**25**	**9.9**	**2550**	**1293**	**8.88**		**25**	**9.7**	**3040**	**1556**	**8.89**		**25**	**9.9**	**2560**	**1300**
Average	**8.63**	**2E-09**	**25**	**9.1**	**1078**	**530**	**8.76**	**2E-09**	**25**	**9.3**	**2367**	**1197**	**8.77**	**2E-09**	**25**	**9.1**	**2516**	**1276**	**8.80**	**2E-09**	**25**	**9.3**	**2431**	**1226**
Stdev	**0.08**		**0.0**	**0.2**	**97**	**50**	**0.08**		**0.0**	**0.3**	**94**	**49**	**0.06**		**0.0**	**0.3**	**242**	**129**	**0.06**		**0.0**	**0.3**	**99**	**52**

ES 4/4/17
ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia*, in a simulated effluent
Test #: Ni WER 1132R CDC
Water Quality Summary

	4.2						6						8.5						12.2					
	NEW						NEW						NEW						NEW					
TEST DAY	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS
0	8.57	2.7E-09	25	8.4	2350	1190	8.56	2.8E-09	25	8.4	2350	1189	8.57	2.7E-09	25	8.4	2350	1189	8.55	2.8E-09	25	8.4	2350	1187
1	8.51	3.1E-09	25	8.4	2330	1175	8.50	3.2E-09	25	8.4	2320	1173	8.50	3.2E-09	25	8.4	2330	1174	8.49	3.2E-09	25	8.4	2320	1172
2	8.29	5.1E-09	25	8.4	2310	1167	8.28	5.2E-09	25	8.4	2310	1167	8.28	5.2E-09	25	8.4	2310	1166	8.28	5.2E-09	25	8.4	2310	1165
3	8.19	6.5E-09	25	8.2	2280	1151	8.16	6.9E-09	25	8.2	2280	1150	8.17	6.8E-09	25	8.2	2280	1150	8.18	6.6E-09	25	8.2	2280	1146
4	8.29	5.1E-09	25	8.6	2250	1139	8.30	5E-09	25	8.5	2260	1141	8.30	5E-09	25	8.5	2250	1139	8.32	4.8E-09	25	8.5	2260	1137
5	8.21	6.2E-09	25	8.6	2300	1164	8.20	6.3E-09	25	8.6	2300	1164	8.22	6E-09	25	8.6	2300	1164	8.22	6E-09	25	8.6	2300	1165
6	8.20	6.3E-09	25	8.7	2320	1175	8.20	6.3E-09	25	8.7	2320	1169	8.19	6.5E-09	25	8.7	2320	1171	8.20	6.3E-09	25	8.6	2310	1168
MIN	**8.19**		**25**	**8.2**	**2250**	**1139**	**8.16**		**25**	**8.2**	**2260**	**1141**	**8.17**		**25**	**8.2**	**2250**	**1139**	**8.18**		**25**	**8.2**	**2260**	**1137**
MAX	**8.57**		**25**	**8.7**	**2350**	**1190**	**8.56**		**25**	**8.7**	**2350**	**1189**	**8.57**		**25**	**8.7**	**2350**	**1189**	**8.55**		**25**	**8.6**	**2350**	**1187**
Average	**8.30**	**5E-09**	**25**	**8.5**	**2306**	**1166**	**8.29**	**5E-09**	**25**	**8.5**	**2306**	**1165**	**8.30**	**5E-09**	**25**	**8.5**	**2306**	**1165**	**8.30**	**5E-09**	**25**	**8.4**	**2304**	**1163**
Stdev	**0.15**		**0.0**	**0.2**	**33**	**17**	**0.16**		**0.0**	**0.2**	**29**	**16**	**0.16**		**0.0**	**0.2**	**33**	**16**	**0.15**		**0.0**	**0.1**	**29**	**17**

	4.2						6						8.5						12.2					
	OLD						OLD						OLD						OLD					
TEST DAY	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS
1	8.80	1.6E-09	25	9.2	2570	1305	8.77	1.7E-09	25	9.4	2640	1339	8.76	1.7E-09	25	9.2	2580	1310	8.75	1.8E-09	25	9.3	2430	1231
2	8.76	1.7E-09	25	9.2	2370	1201	8.73	1.9E-09	25	9.1	2350	1187	8.71	1.9E-09	25	9	2390	1212	8.68	2.1E-09	25	9.2	2280	1152
3	8.80	1.6E-09	25	9	2540	1292	8.82	1.5E-09	25	9	2420	1225	8.80	1.6E-09	25	9	2350	1190	8.75	1.8E-09	25	9.1	2420	1224
4	8.89	1.3E-09	25	9.2	2300	1165	8.90	1.3E-09	25	9.4	2280	1156	8.93	1.2E-09	25	9.5	2300	1160	8.89	1.3E-09	25	9.5	2270	1147
5	8.82	1.5E-09	25	9.8	2330	1177	8.85	1.4E-09	25	9.9	2350	1189	8.85	1.4E-09	25	9.9	2360	1195	8.81	1.5E-09	25	9.9	2320	1171
6	8.78	1.7E-09	25	9.3	2550	1293	8.81	1.5E-09	25	9.4	2410	1218	8.80	1.6E-09	25	9.3	2410	1219	8.79	1.6E-09	25	9.3	2430	1233
7	8.68	2.1E-09	25	9.1	2540	1288	8.70	2E-09	25	9.2	2510	1274	8.73	1.9E-09	25	9.2	2440	1236	8.69	2E-09	25	9.2	2590	1316
MIN	**8.68**		**25**	**9**	**2300**	**1165**	**8.70**		**25**	**9**	**2280**	**1156**	**8.71**		**25**	**9**	**2300**	**1160**	**8.68**		**25**	**9.1**	**2270**	**1147**
MAX	**8.89**		**25**	**9.8**	**2570**	**1305**	**8.90**		**25**	**9.9**	**2640**	**1339**	**8.93**		**25**	**9.9**	**2580**	**1310**	**8.89**		**25**	**9.9**	**2590**	**1316**
Average	**8.79**	**2E-09**	**25**	**9.3**	**2457**	**1246**	**8.79**	**2E-09**	**25**	**9.3**	**2423**	**1227**	**8.79**	**2E-09**	**25**	**9.3**	**2404**	**1217**	**8.76**	**2E-09**	**25**	**9.4**	**2391**	**1211**
Stdev	**0.06**		**0.0**	**0.3**	**118**	**62**	**0.07**		**0.0**	**0.3**	**120**	**62**	**0.08**		**0.0**	**0.3**	**90**	**47**	**0.07**		**0.0**	**0.3**	**112**	**60**

ES 4/4/17
ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia* , in a simulated effluent
Test #: Ni WER 1132R CDC
Water Quality Summary

	17.4					
	NEW					
TEST DAY	pH - New	H+	Temp	DO	Cond	TDS
0	8.57	2.7E-09	25	8.4	2350	1186
1	8.51	3.1E-09	25	8.4	2320	1172
2	8.29	5.1E-09	25	8.4	2300	1164
3	8.18	6.6E-09	25	8.2	2290	1151
4	8.33	4.7E-09	25	8.6	2250	1137
5	8.21	6.2E-09	25	8.6	2300	1164
6	8.21	6.2E-09	25	8.6	2310	1171
MIN	**8.18**		**25**	**8.2**	**2250**	**1137**
MAX	**8.57**		**25**	**8.6**	**2350**	**1186**
Average	**8.31**	**5E-09**	**25**	**8.5**	**2303**	**1164**
Stdev	**0.15**		**0.0**	**0.2**	**30**	**16**

	17.4					
	OLD					
TEST DAY	pH - Old	H+	Temp	DO	Cond	TDS
1	8.74	1.8E-09	25	9.2	2830	1444
2	8.74	1.8E-09	25	9.1	2350	1189
3	8.81	1.5E-09	25	9.1	2290	1157
4	8.90	1.3E-09	25	9.4	2280	1150
5	8.80	1.6E-09	25	9.8	2350	1186
6	8.76	1.7E-09	25	9.3	2410	1218
7	8.69	2E-09	25	9.2	2360	1195
MIN	**8.69**		**25**	**9.1**	**2280**	**1150**
MAX	**8.90**		**25**	**9.8**	**2830**	**1444**
Average	**8.77**	**2E-09**	**25**	**9.3**	**2410**	**1220**
Stdev	**0.07**		**0.0**	**0.2**	**190**	**101**

ES 4/4/17
ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia*, in a simulated effluent with added DOC
Test #: Ni WER 1126 CDC
Water Quality Summary

VHW Concurrent from Ni WER 1125 CDC (no DOC)

	VHW RW (Concurrent)						**DILUTED SIMULATED EFFLUENT/LAB WATER WITH DOC**						**4.5**						**6.5**					
	NEW						NEW						NEW						NEW					
TEST DAY	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS
0	8.54	2.9E-09	25	8.5	991	486	8.56	2.8E-09	25	8.4	2270	1145	8.56	2.8E-09	25	8.4	2270	1148	8.56	2.8E-09	25	8.4	2270	1147
1	8.47	3.4E-09	25	8.2	1014	505	8.54	2.9E-09	25	8.3	2320	1170	8.55	2.8E-09	25	8.3	2330	1180	8.56	2.8E-09	25	8.3	2320	1171
2	8.54	2.9E-09	25	8.6	1003	493	8.67	2.1E-09	25	8.9	2300	1169	8.69	2E-09	25	8.8	2320	1172	8.70	2E-09	25	8.8	2330	1177
3	8.47	3.4E-09	25	8.8	990	485	8.71	1.9E-09	25	8.8	2330	1178	8.74	1.8E-09	25	8.8	2320	1176	8.74	1.8E-09	25	8.8	2330	1177
4	8.46	3.5E-09	25	8.7	1007	494	8.80	1.6E-09	25	8.6	2340	1184	8.80	1.6E-09	25	8.6	2340	1183	8.80	1.6E-09	25	8.6	2340	1183
5	8.44	3.6E-09	25	8.7	998	487	8.68	2.1E-09	25	8.6	2330	1178	8.70	2E-09	25	8.6	2340	1181	8.71	1.9E-09	25	8.5	2340	1180
6	8.38	4.2E-09	25	8.7	946	464	8.68	2.1E-09	25	8.6	2330	1181	8.69	2E-09	25	8.5	2330	1181	8.69	2E-09	25	8.5	2330	1176
7	8.47	3.4E-09	25	8.5	946	464																		
MIN	8.38		25	8.2	946	464	8.54		25	8.3	2270	1145	8.55		25	8.3	2270	1148	8.56		25	8.3	2270	1147
MAX	8.54		25	8.8	1014	505	8.80		25	8.9	2340	1184	8.80		25	8.8	2340	1183	8.80		25	8.8	2340	1183
Average	8.47	3E-09	25	8.6	987	485	8.66	2E-09	25	8.6	2317	1172	8.67	2E-09	25	8.6	2321	1174	8.67	2E-09	25	8.6	2323	1173
Stdev	0.05		0.0	0.2	26	14	0.09		0.0	0.2	24	13	0.09		0.0	0.2	24	12	0.09		0.0	0.2	24	12

	VHW RW (Concurrent)						**DILUTED SIMULATED EFFLUENT/LAB WATER WITH DOC**						**4.5**						**6.5**					
	OLD						OLD						OLD						OLD					
TEST DAY	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS
1	8.60	2.5E-09	25	8.7	1057	517	8.70	2E-09	24	8.7	2370	1198	8.70	2E-09	24	8.5	2410	1217	8.71	1.9E-09	24	8.6	2540	1288
2	8.61	2.5E-09	25	9.1	1040	511	8.78	1.7E-09	25	8.9	2470	1243	8.77	1.7E-09	25	8.9	2470	1333	8.80	1.6E-09	25	9	2510	1253
3	8.66	2.2E-09	25	9.3	1032	507	8.79	1.6E-09	25	9	2370	1200	8.80	1.6E-09	25	9	2750	1399	8.79	1.6E-09	25	8.9	2390	1211
4	8.66	2.2E-09	25	9.1	1105	535	8.81	1.5E-09	25	8.8	2410	1221	8.80	1.6E-09	25	8.6	2430	1228	8.82	1.5E-09	25	8.6	2460	1247
5	8.54	2.9E-09	25	8.6	1026	505	8.73	1.9E-09	25	8.9	2570	1308	8.74	1.8E-09	25	8.8	2630	1337	8.75	1.8E-09	25	8.9	2530	1285
6	8.55	2.8E-09	25	9.2	1025	504	8.64	2.3E-09	25	8.8	2440	1237	8.65	2.2E-09	25	8.8	2710	1374	8.66	2.2E-09	25	8.8	2520	1292
7	8.51	3.1E-09	25	8.7	983	483	8.78	1.7E-09	25	8.6	2440	1265	8.79	1.6E-09	25	8.5	2840	1448	8.78	1.7E-09	25	8.6	2560	1302
8	8.54	2.9E-09	25	8.9	1045	513																		
MIN	8.51		25	8.6	983	483	8.64		24	8.6	2370	1198	8.65		24	8.5	2410	1217	8.66		24	8.6	2390	1211
MAX	8.66		25	9.3	1105	535	8.81		25	9	2570	1308	8.80		25	9	2840	1448	8.82		25	9	2560	1302
Average	8.58	3E-09	25	9.0	1039	509	8.74	2E-09	25	8.8	2439	1239	8.75	2E-09	25	8.7	2606	1334	8.76	2E-09	25	8.8	2501	1268
Stdev	0.06		0.0	0.3	34	15	0.06		0.4	0.1	69	39	0.06		0.4	0.2	171	85	0.06		0.4	0.2	58	32

ES 4/4/17
ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia* , in a simulated effluent with added DOC
Test #: Ni WER 1126 CDC
Water Quality Summary

	9.2						**13.2**						**18.9**					
	NEW						NEW						NEW					
TEST DAY	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS
0	8.56	2.8E-09	25	8.3	2270	1148	8.57	2.7E-09	25	8.3	2270	1147	8.57	2.7E-09	25	8.3	2270	1147
1	8.57	2.7E-09	25	8.2	2320	1170	8.56	2.8E-09	25	8.2	2310	1168	8.56	2.8E-09	25	8.2	2310	1167
2	8.70	2E-09	25	8.7	2330	1175	8.70	2E-09	25	8.7	2330	1175	8.71	1.9E-09	25	8.7	2330	1175
3	8.74	1.8E-09	25	8.8	2330	1176	8.75	1.8E-09	25	8.8	2330	1175	8.75	1.8E-09	25	8.8	2320	1174
4	8.81	1.5E-09	25	8.6	2340	1183	8.80	1.6E-09	25	8.6	2340	1182	8.80	1.6E-09	25	8.6	2340	1182
5	8.72	1.9E-09	25	8.5	2340	1182	8.72	1.9E-09	25	8.5	2340	1181	8.72	1.9E-09	25	8.5	2330	1181
6	8.69	2E-09	25	8.4	2320	1174	8.70	2E-09	25	8.4	2320	1172	8.70	2E-09	25	8.4	2320	1174
7																		
MIN	**8.56**		**25**	**8.2**	**2270**	**1148**	**8.56**		**25**	**8.2**	**2270**	**1147**	**8.56**		**25**	**8.2**	**2270**	**1147**
MAX	**8.81**		**25**	**8.8**	**2340**	**1183**	**8.80**		**25**	**8.8**	**2340**	**1182**	**8.80**		**25**	**8.8**	**2340**	**1182**
Average	**8.68**	**2E-09**	**25**	**8.5**	**2321**	**1173**	**8.68**	**2E-09**	**25**	**8.5**	**2320**	**1171**	**8.68**	**2E-09**	**25**	**8.5**	**2317**	**1171**
Stdev	**0.09**		**0.0**	**0.2**	**24**	**12**	**0.09**		**0.0**	**0.2**	**24**	**12**	**0.09**		**0.0**	**0.2**	**23**	**12**

	9.2						**13.2**						**18.9**					
	OLD						OLD						OLD					
TEST DAY	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS
1	8.73	1.9E-09	24	8.6	2650	1343	8.71	1.9E-09	24	8.6	2430	1233	8.69	2E-09	24	8.5	2360	1193
2	8.81	1.5E-09	25	8.9	2600	1321	8.79	1.6E-09	25	9	2460	1239	8.78	1.7E-09	25	8.9	2450	1238
3	8.80	1.6E-09	25	8.9	2430	1231	8.79	1.6E-09	25	8.9	2380	1203	8.79	1.6E-09	25	8.8	2420	1227
4	8.83	1.5E-09	25	8.7	2550	1300	8.82	1.5E-09	25	8.7	2450	1260	8.81	1.5E-09	25	8.6	2370	1199
5	8.74	1.8E-09	25	8.9	2730	1392	8.75	1.8E-09	25	8.9	2570	1306	8.75	1.8E-09	25	8.9	2590	1314
6	8.67	2.1E-09	25	8.8	2660	1350	8.67	2.1E-09	25	8.8	2490	1263	8.68	2.1E-09	25	8.8	2440	1233
7	8.78	1.7E-09	25	8.6	2560	1301	8.78	1.7E-09	25	8.5	2560	1300	8.79	1.6E-09	25	8.5	2510	1271
8																		
MIN	**8.67**		**24**	**8.6**	**2430**	**1231**	**8.67**		**24**	**8.5**	**2380**	**1203**	**8.68**		**24**	**8.5**	**2360**	**1193**
MAX	**8.83**		**25**	**8.9**	**2730**	**1392**	**8.82**		**25**	**9**	**2570**	**1306**	**8.81**		**25**	**8.9**	**2590**	**1314**
Average	**8.76**	**2E-09**	**25**	**8.8**	**2597**	**1320**	**8.76**	**2E-09**	**25**	**8.8**	**2477**	**1258**	**8.75**	**2E-09**	**25**	**8.7**	**2449**	**1239**
Stdev	**0.06**		**0.4**	**0.1**	**97**	**50**	**0.05**		**0.4**	**0.2**	**69**	**37**	**0.05**		**0.4**	**0.2**	**80**	**42**

ES 4/4/17
ASC 4/4/17

Water Effect Ration (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, Ceriodaphnia dubia, in a simulated effluent with added DOC
Test #: Ni WER 1126 CDC
Water Quality Summary

	26.9						**38.5**					
	NEW						NEW					
TEST DAY	pH - New	H+	Temp	DO	Cond	TDS	pH - New	H+	Temp	DO	Cond	TDS
0	8.56	2.8E-09	25	8.3	2270	1147	8.56	2.8E-09	25	8.3	2270	1147
1	8.56	2.8E-09	25	8.2	2310	1167	8.56	2.8E-09	25	8.2	2310	1167
2	8.71	1.9E-09	25	8.7	2330	1175	8.71	1.9E-09	25	8.7	2330	1175
3	8.74	1.8E-09	25	8.8	2320	1174	8.74	1.8E-09	25	8.8	2310	1174
4	8.81	1.5E-09	25	8.6	2340	1182	8.81	1.5E-09	25	8.6	2340	1182
5	8.72	1.9E-09	25	8.5	2330	1181	8.73	1.9E-09	25	8.4	2330	1181
6	8.70	2E-09	25	8.4	2320	1174	8.70	2E-09	25	8.4	2320	1174
7												
MIN	**8.56**		**25**	**8.2**	**2270**	**1147**	**8.56**		**25**	**8.2**	**2270**	**1147**
MAX	**8.81**		**25**	**8.8**	**2340**	**1182**	**8.81**		**25**	**8.8**	**2340**	**1182**
Average	**8.68**	**2E-09**	**25**	**8.5**	**2317**	**1171**	**8.68**	**2E-09**	**25**	**8.5**	**2316**	**1171**
Stdev	**0.09**		**0.0**	**0.2**	**23**	**12**	**0.09**		**0.0**	**0.2**	**23**	**12**

	26.9						**38.5**					
	OLD						OLD					
TEST DAY	pH - Old	H+	Temp	DO	Cond	TDS	pH - Old	H+	Temp	DO	Cond	TDS
1	8.72	1.9E-09	24	8.5	2650	1349	8.70	2E-09	24	8.5	2370	1198
2	8.81	1.5E-09	25	9	2560	1299	8.79	1.6E-09	25	8.9	2500	1267
3	8.80	1.6E-09	25	8.9	2410	1220	8.81	1.5E-09	25	8.9	2550	1293
4	8.83	1.5E-09	25	8.7	2540	1293	8.81	1.5E-09	25	8.6	2380	1203
5	8.75	1.8E-09	25	8.9	2580	1320	8.75	1.8E-09	25	8.9	2610	1326
6	8.67	2.1E-09	25	8.9	2570	1305	8.69	2E-09	25	8.7	2520	1277
7	8.79	1.6E-09	25	8.5	2590	1318	8.79	1.6E-09	25	8.7	2390	1210
8												
MIN	**8.67**		**24**	**8.5**	**2410**	**1220**	**8.69**		**24**	**8.5**	**2370**	**1198**
MAX	**8.83**		**25**	**9**	**2650**	**1349**	**8.81**		**25**	**8.9**	**2610**	**1326**
Average	**8.76**	**2E-09**	**25**	**8.8**	**2557**	**1301**	**8.76**	**2E-09**	**25**	**8.7**	**2474**	**1253**
Stdev	**0.06**		**0.4**	**0.2**	**73**	**40**	**0.05**		**0.4**	**0.2**	**95**	**50**

ES 4/4/17
ASC 4/4/17

Analytical Report for

OSU Aquatic Toxicology Lab - Dec. 2016#2

ASL Report #: Q3850
Project ID: 921090.OTC

Attn: Allison Cardwell

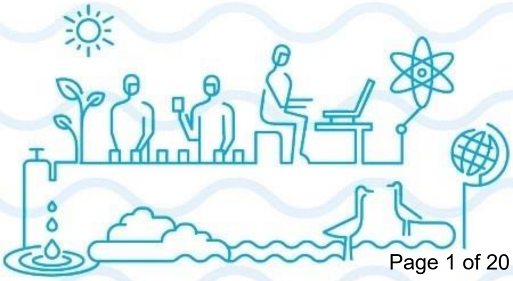
Authorized and Released By:

Emily Biboux

Laboratory Project Manager
Emily Biboux
(541) 758-0235 ext.23118
January 13, 2017

Accredited in accordance with NELAP:
Oregon (100022)
Louisiana (05031)

CH2M – Applied Sciences Laboratory
1100 NE Circle Boulevard, Suite 300
Corvallis, Oregon 97330 Email: asl@ch2m.com
www.ch2m.com



Sample Receipt Comments

We certify that the test results meet all NELAP requirements.

Sample Cross-Reference

ASL Sample ID	Client Sample ID	Date/Time Collected	Date Received
Q385001	Cu 1124_#1 new	12/15/16 17:00	12/27/16
Q385002	Cu 1124_#6 new	12/15/16 17:05	12/27/16
Q385003	Cu 1124_#1 new1	12/22/16 14:00	12/27/16
Q385004	Cu 1124_#1_old	12/22/16 10:45	12/27/16
Q385005	Cu 1124_#2_old	12/22/16 10:50	12/27/16
Q385006	Cu 1124_#3_old	12/22/16 10:55	12/27/16
Q385007	Cu 1124_#4_old	12/22/16 11:00	12/27/16
Q385008	Cu 1124_#5_old	12/22/16 11:10	12/27/16
Q385009	Cu 1124_#6_old	12/22/16 11:15	12/27/16
Q385010	Cu 1124_#7_old	12/22/16 10:40	12/27/16
Q385011	Ni 1126_#1 new	12/19/16 14:45	12/27/16
Q385012	Ni 1125_#1 new	12/19/16 13:50	12/27/16
Q385013	Ni 1126_#7 new	12/21/16 14:30	12/27/16
Q385014	Ni 1126_#8 new	12/21/16 14:35	12/27/16
Q385015	Ni 1126_#7 old	12/22/16 15:00	12/27/16
Q385016	Ni 1126_#8 old	12/22/16 15:05	12/27/16

CASE NARRATIVE
METALS ANALYSIS

Lab Name: CH2M ASL **ASL SDG#:** Q3850

Project: OSU Aquatic Toxicology Lab **Project #:** 921090.OTC

With the exceptions noted as flags, footnotes, or detailed in the section below; standard operating procedures were followed in the analysis of the samples and no problems were encountered or anomalies observed.

All laboratory quality control samples were within established control limits, with any exceptions noted below, or in the associated QC summary forms.

Each sample was analyzed to achieve the lowest possible reporting limit within the constraints of the method. For diluted samples, the reporting limits are adjusted for the dilution required.

Calculations are performed before rounding to minimize errors in calculated values.

All holding times were met and proper preservation noted for the methods performed on these samples, unless otherwise detailed in the section below, or in the sample receipt documentation.

Method(s):
E200.7: E200.2

CH2M ASL

Client Information

Client Sample ID: **Cu 1124_#1 new**

Project Name: OSU Aquatic Toxicology Lab
Sample Date: 12/15/16
Sample Time: 17:00
Type: Grab
Matrix: Water

Lab Information

Lab Sample ID: **Q385001**

Date Received: 12/27/16
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	8140		ug/L	E200.7	E200.2	01/03/17
Magnesium	1	50.0	500	3920		ug/L	E200.7	E200.2	01/03/17
Potassium	1	100	1000	325	J	ug/L	E200.7	E200.2	01/03/17
Sodium	1	250	1000	12600		ug/L	E200.7	E200.2	01/03/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information		**Lab Information**	
Client Sample ID:	**Cu 1124_#6 new**	**Lab Sample ID:**	**Q385002**
Project Name:	OSU Aquatic Toxicology Lab	Date Received:	12/27/16
Sample Date:	12/15/16	Report Revision No:	0
Sample Time:	17:05		
Type:	Grab		
Matrix:	Water		

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	9090		ug/L	E200.7	E200.2	01/03/17
Magnesium	1	50.0	500	4380		ug/L	E200.7	E200.2	01/03/17
Potassium	1	100	1000	402	J	ug/L	E200.7	E200.2	01/03/17
Sodium	1	250	1000	13800		ug/L	E200.7	E200.2	01/03/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information		**Lab Information**	
Client Sample ID:	**Cu 1124_#1 new1**	**Lab Sample ID:**	**Q385003**
Project Name:	OSU Aquatic Toxicology Lab	Date Received:	12/27/16
Sample Date:	12/22/16	Report Revision No:	0
Sample Time:	14:00		
Type:	Grab		
Matrix:	Water		

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	8150		ug/L	E200.7	E200.2	01/03/17
Magnesium	1	50.0	500	3990		ug/L	E200.7	E200.2	01/03/17
Potassium	1	100	1000	300	J	ug/L	E200.7	E200.2	01/03/17
Sodium	1	250	1000	12600		ug/L	E200.7	E200.2	01/03/17

U=Not detected and reported as less than detection limit

J=Estimated value below reporting limit

E=Estimated value above calibration range

*=See case narrative

B=Analyte detected in blank

Client Information

Lab Information

Client Sample ID: **Ni 1126_#1 new**

Lab Sample ID: **Q385011**

Project Name: OSU Aquatic Toxicology Lab
Sample Date: 12/19/16
Sample Time: 14:45
Type: Grab
Matrix: Water

Date Received: 12/27/16
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	46000		ug/L	E200.7	E200.2	01/03/17
Magnesium	1	50.0	500	45800		ug/L	E200.7	E200.2	01/03/17
Potassium	1	100	1000	81900		ug/L	E200.7	E200.2	01/03/17
Sodium	10	2500	10000	393000		ug/L	E200.7	E200.2	01/03/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

CH2M ASL

Client Information

Client Sample ID: **Ni 1125_#1 new**

Project Name: OSU Aquatic Toxicology Lab
Sample Date: 12/19/16
Sample Time: 13:50
Type: Grab
Matrix: Water

Lab Information

Lab Sample ID: **Q385012**

Date Received: 12/27/16
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	13500		ug/L	E200.7	E200.2	01/03/17
Magnesium	1	50.0	500	50800		ug/L	E200.7	E200.2	01/03/17
Potassium	1	100	1000	80700		ug/L	E200.7	E200.2	01/03/17
Sodium	10	2500	10000	388000		ug/L	E200.7	E200.2	01/03/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information

Lab Information

Method Blank ID: WB1-1230

Project Name: OSU Aquatic Toxicology Lab
Sample Date: N/A
Sample Time: N/A
Type: QC
Matrix: Water

Date Received: N/A
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	200	U	ug/L	E200.7	E200.2	01/03/17
Magnesium	1	50.0	500	50.0	U	ug/L	E200.7	E200.2	01/03/17
Potassium	1	100	1000	100	U	ug/L	E200.7	E200.2	01/03/17
Sodium	1	250	1000	250	U	ug/L	E200.7	E200.2	01/03/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

CH2M ASL

Client Information

Project Name: OSU Aquatic Toxicology Lab
Type: QC
Matrix: Water

Lab Information

Blank Spike ID: BS1W1230

Report Revision No: 0
Dilution Factor: 1

Analyte	Spike Amount	Result	Units	%Recovery	Analysis Method	Prep Method	Date Analyzed
Metals							
Calcium	10000	9790	ug/L	98	E200.7	E200.2	01/03/17
Magnesium	10000	10200	ug/L	102	E200.7	E200.2	01/03/17
Potassium	5000	4910	ug/L	98	E200.7	E200.2	01/03/17
Sodium	10000	10400	ug/L	104	E200.7	E200.2	01/03/17

U=Not detected and report as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

CASE NARRATIVE
GENERAL CHEMISTRY ANALYSIS

Lab Name: CH2M ASL **ASL SDG#:** Q3850

Project: OSU Aquatic Toxicology Lab **Project #:** 921090.OTC

With the exceptions noted as flags, footnotes, or detailed in the section below; standard operating procedures were followed in the analysis of the samples and no problems were encountered or anomalies observed.

All laboratory quality control samples were within established control limits, with any exceptions noted below, or in the associated QC summary forms.

Each sample was analyzed to achieve the lowest possible reporting limit within the constraints of the method. For diluted samples, the reporting limits are adjusted for the dilution required.

Calculations are performed before rounding to minimize errors in calculated values.

All holding times were met and proper preservation noted for the methods performed on these samples, unless otherwise detailed in the section below, or in the sample receipt documentation.

Method(s):
E300.0A

CH2M ASL

Client Information

Project Name: **OSU Aquatic Toxicology Lab**

Date Received: 12/27/16
Type: See C.O.C.
Matrix: Water

Lab Information

Lab Batch ID: **Q3850**

Analysis Method: E300.0A
Units: mg/L
Report Revision No.: 0

Client Sample ID	**Lab Sample ID**	**Dilution Factor**	**Chloride** **DL**	**RL**	**Result**	**Qualifier**	**Date Analyzed**
General Chemistry							
Cu 1124_#1 new	Q385001	5	0.10	1.00	39.9		12/29/16
Cu 1124_#6 new	Q385002	10	0.20	2.00	46.8		12/29/16
Cu 1124_#1 new1	Q385003	10	0.20	2.00	43.1		12/29/16
Ni 1126_#1 new	Q385011	100	2.00	20.0	349		12/29/16
Ni 1125_#1 new	Q385012	100	2.00	20.0	348		12/29/16
WB1-1229	WB1-1229	1	0.020	0.20	0.020	U	12/29/16

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

CH2M ASL

Client Information		**Lab Information**	
Project Name:	**OSU Aquatic Toxicology Lab**	**Lab Batch ID:**	**Q3850**
Date Received:	12/27/16	Analysis Method:	E300.0A
Type:	See C.O.C.	Units:	mg/L
Matrix:	Water	Report Revision No.:	0

Client Sample ID	Lab Sample ID	Dilution Factor	Sulfate DL	Sulfate RL	Sulfate Result	Sulfate Qualifier	Date Analyzed
General Chemistry							
Cu 1124_#1 new	Q385001	1	0.023	0.20	0.46		01/04/17
Cu 1124_#6 new	Q385002	1	0.023	0.20	0.69		01/04/17
Cu 1124_#1 new1	Q385003	1	0.023	0.20	0.46		01/04/17
Ni 1126_#1 new	Q385011	100	2.27	20.0	321		12/29/16
Ni 1125_#1 new	Q385012	100	2.27	20.0	322		12/29/16
WB1-0104	WB1-0104	1	0.023	0.20	0.023	U	01/04/17
WB1-1229	WB1-1229	1	0.023	0.20	0.088	J	12/29/16

U=Not detected and reported as less than detection limit

J=Estimated value below reporting limit

E=Estimated value above calibration range

*=See case narrative

B=Analyte detected in blank

Client Information

Lab Information

Lab Batch ID: Q3850

Project Name: OSU Aquatic Toxicology Lab
Type: QC
Matrix: Water

Report Revision No.: 0

LCS ID	Analyte	Spike Amount	Sample Result	Units	% Recovery	Analysis Method	Date Analyzed
General Chemistry							
BS1W1229	Chloride	5.00	5.05	mg/L	101	E300.0A	12/29/16
BS1W1229	Sulfate	5.00	4.94	mg/L	99	E300.0A	12/29/16
BS1W0104	Sulfate	5.00	4.89	mg/L	98	E300.0A	01/04/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

CASE NARRATIVE
GENERAL CHEMISTRY ANALYSIS

Lab Name: CH2M ASL

ASL SDG#: Q3850

Project: OSU Aquatic Toxicology Lab

Project #: 921090.OTC

With the exceptions noted as flags, footnotes, or detailed in the section below; standard operating procedures were followed in the analysis of the samples and no problems were encountered or anomalies observed.

All laboratory quality control samples were within established control limits, with any exceptions noted below, or in the associated QC summary forms.

Each sample was analyzed to achieve the lowest possible reporting limit within the constraints of the method. For diluted samples, the reporting limits are adjusted for the dilution required.

Calculations are performed before rounding to minimize errors in calculated values.

All holding times were met and proper preservation noted for the methods performed on these samples, unless otherwise detailed in the section below, or in the sample receipt documentation.

Method(s):
SM5310B

CH2M ASL

Client Information		**Lab Information**	
Project Name:	**OSU Aquatic Toxicology Lab**	**Lab Batch ID:**	**Q3850**
Date Received:	12/27/16	Analysis Method:	SM5310B
Type:	See C.O.C.	Units:	mg/L
Matrix:	Water	Report Revision No.:	0

Client Sample ID	Lab Sample ID	Dilution Factor	DL	Dissolved Organic Carbon RL	Result	Qualifier	Date Analyzed
General Chemistry							
Cu 1124_#1 new	Q385001	1	0.20	0.50	1.51		01/09/17
Cu 1124_#6 new	Q385002	1	0.20	0.50	1.34		01/09/17
Cu 1124_#1 new1	Q385003	1	0.20	0.50	1.44		01/09/17
Cu 1124_#1_old	Q385004	1	0.20	0.50	1.36		01/09/17
Cu 1124_#2_old	Q385005	1	0.20	0.50	1.38		01/09/17
Cu 1124_#3_old	Q385006	1	0.20	0.50	1.34		01/11/17
Cu 1124_#4_old	Q385007	1	0.20	0.50	1.28		01/11/17
Cu 1124_#5_old	Q385008	1	0.20	0.50	1.25		01/11/17
Cu 1124_#6_old	Q385009	1	0.20	0.50	1.22		01/11/17
Cu 1124_#7_old	Q385010	1	0.20	0.50	0.20	U	01/11/17
Ni 1126_#1 new	Q385011	1	0.20	0.50	11.2		01/11/17
Ni 1125_#1 new	Q385012	1	0.20	0.50	0.22	J	01/12/17
Ni 1126_#7 new	Q385013	1	0.20	0.50	11.3		01/12/17
Ni 1126_#8 new	Q385014	1	0.20	0.50	11.4		01/12/17
Ni 1126_#7 old	Q385015	1	0.20	0.50	14.0		01/12/17
Ni 1126_#8 old	Q385016	1	0.20	0.50	13.1		01/12/17
WB1-0109	WB1-0109	1	0.20	0.50	0.20	U	01/09/17
WB1-0111	WB1-0111	1	0.20	0.50	0.20	U	01/11/17

U=Not detected and reported as less than detection limit

B=Analyte detected in blank

J=Estimated value below reporting limit

E=Estimated value above calibration range

*=See case narrative

Client Information

Lab Information

Lab Batch ID: Q3850

Project Name: OSU Aquatic Toxicology Lab

Type: QC

Matrix: Water

Report Revision No.: 0

LCS ID	Analyte	Spike Amount	Sample Result	Units	% Recovery	Analysis Method	Date Analyzed
General Chemistry							
BS1W0109	Dissolved Organic Carbon	5.00	4.91	mg/L	98	SM5310B	01/09/17
BS1W0111	Dissolved Organic Carbon	5.00	4.79	mg/L	96	SM5310B	01/11/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

COC #1 of 2

CH2MHILL Applied Sciences Laboratory
CHAIN OF CUSTODY RECORD
AND AGREEMENT TO PERFORM SERVICES

Chain of Custody Record

1100 NE Circle Blvd. Suite 300
Corvallis, OR 97330
(541) 768-3120

Client Contact

Project Name: OSU_Dec. 2016 #2
Project # or PO #: –
Company Name: OSU Aquatic Tox Lab
Address: 33972 Texas St. SW
City/State/Zip: Albany, OR 97321
Project Manager: Allison Cardwell
Phone #: 541-926-1254
Report to email: allison.cardwell@oregonstate.edu

Analysis Turnaround Time

TAT is Calander days

TAT if different from below ______

☒ 21 days (STD)
☐ 14 days *
☐ 7 days *
☐ 5 days *
☐ 3 day *
☐ 2 days *
☐ 1 day *

* (Surcharges will apply)

For Lab Use Only:

SDG: Q3850
Custody Seals Intact? ☐ Yes ☒ No
Hand delivered? ☒ Yes ☐ No
Cooler Temp 6.4 °C
Therm ID No.: 173 Therm Exp. 1/18/17
Packing Material: Circle Below
Ice [✓ Blue Ice] Box Bubble Wrap
Radiological Screen? ☐ Yes ☒ No

Sample Identification (Limit of 20 characters)	Sample Date	Sample Time	Sample Type (C=Comp, G=Grab)	Matrix (Water, Soil, Air)	Total # of Cont.	Ca, Mg, Na, K	Cl⁻, SO4	DOC							Sample Specific Notes:	Lab ID:
Preservation Used						4	1	1								
Cu 1124_#1 new	12/15/16	1700	G	H_2O	3	X	X	X								1
Cu 1124_#6 new	12/15/16	1705	G	H_2O	3	X	X	X								2
Cu 1124_#1 new1	12/22/16	1400	G	H_2O	3	X	X	X								3
Cu 1124_#1_old	12/22/16	1045	G	H_2O	1			X								4
Cu 1124_#2_old	12/22/16	1050	G	H_2O	1			X								5
Cu 1124_#3_old	12/22/16	1055	G	H_2O	1			X								6
Cu 1124_#4_old	12/22/16	1100	G	H_2O	1			X								7
Cu 1124_#5_old	12/22/16	1110	G	H_2O	1			X								8
Cu 1124_#6_old	12/22/16	1115	G	H_2O	1			X								9
Cu 1124_#7_old	12/22/16	1040	G	H_2O	1			X								10
Ni 1126_#1 new	12/19/16	1445	G	H_2O	3	X	X	X								11
Ni 1125_#1 new	12/19/16	1350	G	H_2O	3	X	X	X								12
Ni 1126_#7 new	12/21/16 ~~12/22/16~~	1430	G	H_2O	1			X								13

Preservation Used: 1= Ice, 2= HCl; 3= H2SO4; 4=HNO3; 5=NaOH; 6= Other ______

Possible Hazard Identification:
Are samples hazardous? ☐ Yes ☒ No
If yes, select hazard(s): ☐ Listed ☐ Ignitable ☐ Corrosive ☐ Reactive ☐ Toxic
If YES or NO is not checked above, samples will be assumed hazardous and hazardous disposal fees will be applied.

Sample Disposal (A fee may be added if samples are retained longer than 30 day per client request, samples are returned to client, or classified as hazardous.)
☐ Return to Client ☒ Disposal by Lab ☐ Archive for ______ months

Sampled By: Allison Cardwell Allison Cardwell Date/Time: 12/27/16 @ 1330
Relinquished by: Date/Time:
Received by: Date/Time:
Relinquished by: Date/Time: 12/27/16 1400
Received in Laboratory by: Karin Odell Date/Time: 12/27/16 1401
Shipped Via: ☐ UPS ☐ Fed-Ex ☐ USPS ☒ Other Tracking #:

Special Instructions/QC Requirements
If any samples are outside of holding time or temperature → proceed with analysis

COC #2 of 2

CH2MHILL Applied Sciences Laboratory
CHAIN OF CUSTODY RECORD
AND AGREEMENT TO PERFORM SERVICES

Chain of Custody Record

1100 NE Circle Blvd. Suite 300
Corvallis, OR 97330
(541) 768-3120

Client Contact

Project Name: OSU_Dec. 2016 #2
Project # or PO #: –
Company Name: OSU Aquatic Tox Lab
Address: 33972 Texas St. SW
City/State/Zip: Albany, OR 97321
Project Manager: Allison Cardwell
Phone #: 541-926-1254
Report to email: allison.cardwell@oregonstate.edu

Analysis Turnaround Time

TAT is Calander days

TAT if different from below ______

☒ 21 days (STD)
☐ 14 days * ☐ 3 day *
☐ 7 days * ☐ 2 days *
☐ 5 days * ☐ 1 day *

* (Surcharges will apply)

For Lab Use Only:

SDG: Q3850
Custody Seals intact? ☐ Yes ☒ No
Hand delivered? ☒ Yes ☐ No
Cooler Temp: 6.4 C
Therm ID No.: 173 Therm Exp. 1/17/17
Packing Material: Circle Below
Ice [✓ Blue Ice] Box Bubble Wrap
Radiological Screen? ☐ Yes ☒ No

Sample Identification (Limit of 20 characters)	Sample Date	Sample Time	Sample Type (C=Comp, G=Grab)	Matrix (Water, Soil, Air)	Total # of Cont.	DOC	Sample Specific Notes:	Lab ID:
Preservation Used						1		
Ni 1126_#8 new	12/21/16	1435	G	H2O	1	X		14
Ni 1126_#7 old	12/22/16	1500	G	H2O	1	X		15
Ni 1126_#8 old	12/22/16	1505	G	H2O	1	X		16

Preservation Used: 1= Ice, 2= HCl; 3= H2SO4; 4=HNO3; 5=NaOH; 6= Other ______

Possible Hazard Identification:
Are samples hazardous? ☐ Yes ☒ No
If yes, select hazard(s): ☐ Listed ☐ Ignitable ☐ Corrosive ☐ Reactive ☐ Toxic
If YES or NO is not checked above, samples will be assumed hazardous and hazardous disposal fees will be applied.

Sample Disposal (A fee may be added if samples are retained longer than 30 day per client request, samples are returned to client, or classified as hazardous.)
☐ Return to Client ☒ Disposal by Lab ☐ Archive for ______ months

Sampled By: Allison Cardwell Allison Cardwell Date/Time: 12/27/16 @ 1330
Received by: Date/Time:
Received in Laboratory by: Katie Odell Date/Time: 12/27/16 1401

Relinquished by: Date/Time:
Relinquished by: Date/Time: 12/27/16 1400
Shipped Via: ☐ UPS ☐ Fed-Ex ☐ USPS ☒ Other Tracking #:

Special Instructions/QC Requirements
If any samples are outside of temperature or holding time → proceed with analysis

CH2M Applied Sciences Laboratory

Sample Receipt Record

SDG ID: Q3850 Date Received: 12/27/2016

Client/Project: OSU Aquatic Tox Received by: PC

Were custody seals intact and on the outside of the cooler?	☐ Yes	☐ No	☑ N/A
Shipping Record:	☑ Hand Delivered	☐ On File	☐ COC
Radiological Screening for DoD	☐ Yes	☐ No	☑ N/A
Packing Material:	☑ Hand Delivered	☐ Ice ☐ Blue Ice	☐ Box
Temp OK? (<6C) Therm ID: TH173 Exp. 1/17/17 6.4°C	☐ Yes	☐ No	☑ N/A
Was a Chain of Custody (CoC) Provided?	☑ Yes	☐ No	☐ N/A
Was the CoC correctly filled out (If No, document below)	☑ Yes	☐ No	☐ N/A
Did sample labels agree with COC? (If No, document below)	☑ Yes	☐ No	☐ N/A
Did the CoC list a correct bottle count and the preservative types (No=Correct on CoC)	☑ Yes	☐ No	☐ N/A
Were the sample containers in good condition (not broken or leaking)?	☑ Yes	☐ No	☐ N/A
Was enough sample volume provided for analysis? (If No, document below)	☑ Yes	☐ No	☐ N/A
Containers supplied by ASL?	☑ Yes	☐ No	☐ N/A
Any sample with < 1/2 holding time remaining? If so contact LPM and document below.	☑ Yes	☐ No	☐ N/A
Samples have multi-phase? If yes, document on SRER	☐ Yes	☑ No	☐ N/A
All water VOCs free of air bubbles? No, document on SRER	☐ Yes	☐ No	☑ N/A
pH of all samples met criteria on receipt? If "No", preserve and document below.	☑ Yes	☐ No	☐ N/A
Dissolved/Soluble metals filtered in the field?	☐ Yes	☐ No	☑ N/A
Dissolved/Soluble metals have sediment in bottom of container? If so document below.	☐ Yes	☐ No	☑ N/A

Preservation Adjustment

Sample ID	Reagent	Reagent Lot Number	Volume Added	Initials/Date-Time	24 hour pH check Initials/Time

Did pH of all metals samples preserved upon receipt meet criteria 24 hours after preservation? ☐ Yes ☐ No

Sample Exception Report (The following exceptions were noted)

1. All samples for DOC filtered upon arrival into 8oz polys with 1mL 50% H2SO4 (lot#1020) using Whatman 0.45 filters (lot#G9968159).
2. All DOC samples filtered/preserved passed holding time.

Client was notified on: Client contact:

Resolution to Exception:

2. Proceed per COC.

Analytical Report for

OSU Aquatic Toxicology Lab - OSU_January 2017

ASL Report #: R1113

Project ID: 921090.OTC

Attn: Allison Cardwell

Authorized and Released By:

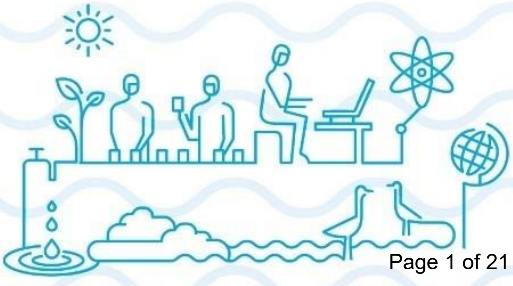
Emily Biboux

Laboratory Project Manager
Emily Biboux
(541) 758-0235 ext.23118
February 07, 2017

All analyses performed by CH2M HILL are clearly indicated. Any subcontracted analyses are included as appended reports as received from the subcontracted laboratory. The results included in this report only relate to the samples listed on the following Sample Cross-Reference page. This report shall not be reproduced except in full, without the written approval of the laboratory.

Accredited in accordance with NELAP:
Oregon (100022)
Louisiana (05031)

Any unusual difficulties encountered during the analysis of your samples are discussed in the attached case narratives.



Sample Receipt Comments

We certify that the test results meet all NELAP requirements.

Sample Cross-Reference

ASL Sample ID	Client Sample ID	Date/Time Collected	Date Received
R111301	Ni 1132R #1 new	01/12/17 10:00	01/19/17
R111302	Ni 1132R #8 new	01/12/17 10:05	01/19/17
R111303	Ni 1132R #1 old	01/13/17 11:00	01/19/17
R111304	Ni 1132R #8 old	01/13/17 11:05	01/19/17
R111305	Paulina_LM1/2	01/18/17 12:00	01/19/17
R111306	Paulina_1 filt	01/19/17 09:00	01/19/17
R111307	Paulina_1 filt-PP	01/19/17 12:00	01/19/17
R111308	OSU Effluent	01/19/17 12:15	01/19/17

CASE NARRATIVE
METALS ANALYSIS

Lab Name: CH2M ASL **ASL SDG#:** R1113

Project: OSU Aquatic Toxicology Lab **Project #:** 921090.OTC

With the exceptions noted as flags, footnotes, or detailed in the section below; standard operating procedures were followed in the analysis of the samples and no problems were encountered or anomalies observed.

All laboratory quality control samples were within established control limits, with any exceptions noted below, or in the associated QC summary forms.

Each sample was analyzed to achieve the lowest possible reporting limit within the constraints of the method. For diluted samples, the reporting limits are adjusted for the dilution required.

Calculations are performed before rounding to minimize errors in calculated values.

All holding times were met and proper preservation noted for the methods performed on these samples, unless otherwise detailed in the section below, or in the sample receipt documentation.

Method(s):
E200.7: E200.2
E200.8: E200.2

Client Information		Lab Information	
Client Sample ID:	**Ni 1132R #1 new**	**Lab Sample ID:**	**R111301**
Project Name:	OSU Aquatic Toxicology Lab	Date Received:	01/19/17
Sample Date:	01/12/17	Report Revision No:	0
Sample Time:	10:00		
Type:	Grab		
Matrix:	Water		

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	36400		ug/L	E200.7	E200.2	01/24/17
Magnesium	1	50.0	500	46900		ug/L	E200.7	E200.2	01/24/17
Potassium	1	100	1000	86000		ug/L	E200.7	E200.2	01/24/17
Sodium	10	2500	10000	379000		ug/L	E200.7	E200.2	01/24/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative
B=Analyte detected in blank

CH2M ASL

Client Information		**Lab Information**	
Client Sample ID:	**Paulina_LM1/2**	**Lab Sample ID:**	**R111305**
Project Name:	OSU Aquatic Toxicology Lab	Date Received:	01/19/17
Sample Date:	01/18/17	Report Revision No:	0
Sample Time:	12:00		
Type:	Grab		
Matrix:	Water		

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	32500		ug/L	E200.7	E200.2	01/24/17
Magnesium	1	50.0	500	45200		ug/L	E200.7	E200.2	01/24/17
Potassium	1	100	1000	7620		ug/L	E200.7	E200.2	01/24/17
Sodium	1	250	1000	66900		ug/L	E200.7	E200.2	01/24/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information

Client Sample ID: **Paulina_1 filt**

Project Name: OSU Aquatic Toxicology Lab
Sample Date: 01/19/17
Sample Time: 09:00
Type: Grab
Matrix: Water

Lab Information

Lab Sample ID: **R111306**

Date Received: 01/19/17
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Calcium	1	200	500	27800		ug/L	E200.7	E200.2	01/24/17
Magnesium	1	50.0	500	41900		ug/L	E200.7	E200.2	01/24/17
Potassium	1	100	1000	5670		ug/L	E200.7	E200.2	01/24/17
Sodium	1	250	1000	50400		ug/L	E200.7	E200.2	01/24/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information | **Lab Information**

Client Sample ID: Paulina_1 filt-PP

Lab Sample ID: R111307

Project Name: OSU Aquatic Toxicology Lab
Sample Date: 01/19/17
Sample Time: 12:00
Type: Grab
Matrix: Water

Date Received: 01/19/17
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Aluminum	1	3.17	10.0	3.17	U	ug/L	E200.8	E200.2	01/23/17
Antimony	1	0.031	0.50	0.031	U	ug/L	E200.8	E200.2	01/23/17
Arsenic	1	0.030	0.50	12.7		ug/L	E200.8	E200.2	01/23/17
Beryllium	1	0.025	0.50	0.025	U	ug/L	E200.8	E200.2	01/23/17
Cadmium	1	0.030	0.50	0.032	J	ug/L	E200.8	E200.2	01/23/17
Chromium	1	0.10	1.00	0.15	J	ug/L	E200.8	E200.2	01/23/17
Copper	1	0.50	2.00	0.50	U	ug/L	E200.8	E200.2	01/23/17
Iron	1	10.0	100	10.0	U	ug/L	E200.7	E200.2	01/24/17
Lead	1	0.041	0.50	0.041	U	ug/L	E200.8	E200.2	01/23/17
Nickel	1	0.025	0.50	1.71		ug/L	E200.8	E200.2	01/23/17
Selenium	1	0.069	1.00	0.089	J	ug/L	E200.8	E200.2	01/23/17
Silver	1	0.025	0.50	0.025	U	ug/L	E200.8	E200.2	01/23/17
Zinc	1	2.50	10.0	2.50	U	ug/L	E200.8	E200.2	01/23/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

CH2M ASL

Client Information

Project Name: OSU Aquatic Toxicology Lab
Sample Date: N/A
Sample Time: N/A
Type: QC
Matrix: Water

Lab Information

Method Blank ID: WB1-0120

Date Received: N/A
Report Revision No: 0

Analyte	Dilution Factor	DL	RL	Result	Qual	Units	Analysis Method	Prep Method	Date Analyzed
Metals									
Aluminum	1	3.17	10.0	3.17	U	ug/L	E200.8	E200.2	01/23/17
Antimony	1	0.031	0.50	0.031	U	ug/L	E200.8	E200.2	01/23/17
Arsenic	1	0.030	0.50	0.030	U	ug/L	E200.8	E200.2	01/23/17
Beryllium	1	0.025	0.50	0.025	U	ug/L	E200.8	E200.2	01/23/17
Cadmium	1	0.030	0.50	0.030	U	ug/L	E200.8	E200.2	01/23/17
Calcium	1	200	500	200	U	ug/L	E200.7	E200.2	01/24/17
Chromium	1	0.10	1.00	0.10	U	ug/L	E200.8	E200.2	01/23/17
Copper	1	0.50	2.00	0.50	U	ug/L	E200.8	E200.2	01/23/17
Iron	1	10.0	100	10.0	U	ug/L	E200.7	E200.2	01/24/17
Lead	1	0.041	0.50	0.041	U	ug/L	E200.8	E200.2	01/23/17
Magnesium	1	50.0	500	50.0	U	ug/L	E200.7	E200.2	01/24/17
Nickel	1	0.025	0.50	0.026	J	ug/L	E200.8	E200.2	01/23/17
Potassium	1	100	1000	100	U	ug/L	E200.7	E200.2	01/24/17
Selenium	1	0.069	1.00	0.069	U	ug/L	E200.8	E200.2	01/23/17
Silver	1	0.025	0.50	0.025	U	ug/L	E200.8	E200.2	01/23/17
Sodium	1	250	1000	250	U	ug/L	E200.7	E200.2	01/24/17
Zinc	1	2.50	10.0	2.50	U	ug/L	E200.8	E200.2	01/23/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information

Project Name: OSU Aquatic Toxicology Lab
Type: QC
Matrix: Water

Lab Information

Blank Spike ID: BS1W0120

Report Revision No: 0
Dilution Factor: 1

Analyte	Spike Amount	Result	Units	%Recovery	Analysis Method	Prep Method	Date Analyzed
Metals							
Aluminum	50.0	44.4	ug/L	89	E200.8	E200.2	01/23/17
Antimony	50.0	51.7	ug/L	103	E200.8	E200.2	01/23/17
Arsenic	50.0	50.6	ug/L	101	E200.8	E200.2	01/23/17
Beryllium	50.0	50.3	ug/L	101	E200.8	E200.2	01/23/17
Cadmium	50.0	50.8	ug/L	102	E200.8	E200.2	01/23/17
Calcium	10000	9910	ug/L	99	E200.7	E200.2	01/24/17
Chromium	50.0	47.1	ug/L	94	E200.8	E200.2	01/23/17
Copper	50.0	45.4	ug/L	91	E200.8	E200.2	01/23/17
Iron	500	536	ug/L	107	E200.7	E200.2	01/24/17
Lead	50.0	46.7	ug/L	93	E200.8	E200.2	01/23/17
Magnesium	10000	10700	ug/L	107	E200.7	E200.2	01/24/17
Nickel	50.0	50.4	ug/L	101	E200.8	E200.2	01/23/17
Potassium	5000	5070	ug/L	101	E200.7	E200.2	01/24/17
Selenium	50.0	49.6	ug/L	99	E200.8	E200.2	01/23/17
Silver	25.0	25.3	ug/L	101	E200.8	E200.2	01/23/17
Sodium	10000	10600	ug/L	106	E200.7	E200.2	01/24/17
Zinc	50.0	50.7	ug/L	101	E200.8	E200.2	01/23/17

U=Not detected and report as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

CASE NARRATIVE
GENERAL CHEMISTRY ANALYSIS

Lab Name: CH2M ASL **ASL SDG#:** R1113

Project: OSU Aquatic Toxicology Lab **Project #:** 921090.OTC

With the exceptions noted as flags, footnotes, or detailed in the section below; standard operating procedures were followed in the analysis of the samples and no problems were encountered or anomalies observed.

All laboratory quality control samples were within established control limits, with any exceptions noted below, or in the associated QC summary forms.

Each sample was analyzed to achieve the lowest possible reporting limit within the constraints of the method. For diluted samples, the reporting limits are adjusted for the dilution required.

Calculations are performed before rounding to minimize errors in calculated values.

All holding times were met and proper preservation noted for the methods performed on these samples, unless otherwise detailed in the section below, or in the sample receipt documentation.

Method(s):
E300.0A

Client Information		**Lab Information**	
Project Name:	**OSU Aquatic Toxicology Lab**	**Lab Batch ID:**	**R1113**
Date Received:	01/19/17	Analysis Method:	E300.0A
Type:	See C.O.C.	Units:	mg/L
Matrix:	Water	Report Revision No.:	0

Client Sample ID	**Lab Sample ID**	**Dilution Factor**	**Chloride** **DL**	**RL**	**Result**	**Qualifier**	**Date Analyzed**
General Chemistry							
Ni 1132R #1 new	R111301	100	2.00	20.0	348		01/26/17
Paulina_LM1/2	R111305	1	0.020	0.20	12.7		01/23/17
Paulina_1 filt	R111306	1	0.020	0.20	3.59		01/23/17
WB1-0123	WB1-0123	1	0.020	0.20	0.020	U	01/23/17
WB1-0126	WB1-0126	1	0.020	0.20	0.020	U	01/26/17

U=Not detected and reported as less than detection limit

B=Analyte detected in blank

J=Estimated value below reporting limit

E=Estimated value above calibration range

*=See case narrative

CH2M ASL

Client Information		**Lab Information**	
Project Name:	**OSU Aquatic Toxicology Lab**	**Lab Batch ID:**	**R1113**
Date Received:	01/19/17	Analysis Method:	E300.0A
Type:	See C.O.C.	Units:	mg/L
Matrix:	Water	Report Revision No.:	0

Client Sample ID	**Lab Sample ID**	**Dilution Factor**	**DL**	**Sulfate** **RL**	**Result**	**Qualifier**	**Date Analyzed**
General Chemistry							
Ni 1132R #1 new	R111301	100	2.27	20.0	316		01/26/17
Paulina_LM1/2	R111305	1	0.023	0.20	16.6		01/23/17
Paulina_1 filt	R111306	1	0.023	0.20	2.99		01/23/17
WB1-0123	WB1-0123	1	0.023	0.20	0.023	U	01/23/17
WB1-0126	WB1-0126	1	0.023	0.20	0.023	U	01/26/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information

Lab Information

Lab Batch ID: R1113

Project Name: OSU Aquatic Toxicology Lab
Type: QC
Matrix: Water

Report Revision No.: 0

LCS ID	Analyte	Spike Amount	Sample Result	Units	% Recovery	Analysis Method	Date Analyzed
General Chemistry							
BS1W0123	Chloride	5.00	4.74	mg/L	95	E300.0A	01/23/17
BS1W0123	Sulfate	5.00	4.65	mg/L	93	E300.0A	01/23/17
BS1W0126	Chloride	5.00	4.92	mg/L	98	E300.0A	01/26/17
BS1W0126	Sulfate	5.00	4.88	mg/L	98	E300.0A	01/26/17

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E=Estimated value above calibration range
*=See case narrative

CASE NARRATIVE
GENERAL CHEMISTRY ANALYSIS

Lab Name: CH2M ASL

ASL SDG#: R1113

Project: OSU Aquatic Toxicology Lab

Project #: 921090.OTC

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Method(s):
SM5310B

CH2M ASL

Client Information		**Lab Information**	
Project Name:	**OSU Aquatic Toxicology Lab**	**Lab Batch ID:**	**R1113**
Date Received:	01/19/17	Analysis Method:	SM5310B
Type:	See C.O.C.	Units:	mg/L
Matrix:	Water	Report Revision No.:	0

Client Sample ID	Lab Sample ID	Dilution Factor	Dissolved Organic Carbon DL	RL	Result	Qualifier	Date Analyzed
General Chemistry							
Ni 1132R #1 new	R111301	1	0.20	0.50	0.20	U	02/02/17
Ni 1132R #8 new	R111302	1	0.20	0.50	0.20	U	02/02/17
Ni 1132R #1 old	R111303	1	0.20	0.50	0.93		02/02/17
Ni 1132R #8 old	R111304	1	0.20	0.50	1.02		02/02/17
Paulina_LM1/2	R111305	1	0.20	0.50	0.87		02/02/17
Paulina_1 filt	R111306	1	0.20	0.50	0.70		02/02/17
WB1-0201	WB1-0201	1	0.20	0.50	0.20	U	02/01/17

U=Not detected and reported as less than detection limit

J=Estimated value below reporting limit

E=Estimated value above calibration range

*=See case narrative

B=Analyte detected in blank

Client Information		**Lab Information**
		Lab Batch ID: R1113
Project Name:	OSU Aquatic Toxicology Lab	Report Revision No.: 0
Type:	QC	
Matrix:	Water	

LCS ID	Analyte	Spike Amount	Sample Result	Units	% Recovery	Analysis Method	Date Analyzed
General Chemistry							
BS1W0201	Dissolved Organic Carbon	5.00	4.58	mg/L	92	SM5310B	02/01/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

CASE NARRATIVE
GENERAL CHEMISTRY ANALYSIS

Lab Name: CH2M ASL **ASL SDG#:** R1113

Project: OSU Aquatic Toxicology Lab **Project #:** 921090.OTC

With the exceptions noted as flags, footnotes, or detailed in the section below; standard operating procedures were followed in the analysis of the samples and no problems were encountered or anomalies observed.

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All holding times were met and proper preservation noted for the methods performed on these samples, unless otherwise detailed in the section below, or in the sample receipt documentation.

Method(s):
SM5210B

Client Information

Project Name: **OSU Aquatic Toxicology Lab**

Date Received: 01/19/17
Type: See C.O.C.
Matrix: Water

Lab Information

Lab Batch ID: **R1113**

Analysis Method: SM5210B
Units: mg/L
Report Revision No.: 0

			BOD5				
Client Sample ID	**Lab Sample ID**	**Dilution Factor**	**DL**	**RL**	**Result**	**Qualifier**	**Date Analyzed**
General Chemistry							
OSU Effluent	R111308	1	N/A	2.0	16.2		01/19/17 16:22
WB1-0119	WB1-0119	1	N/A	2.0	2.0	U	01/19/17 15:36

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

B=Analyte detected in blank

Client Information		**Lab Information**
		Lab Batch ID: R1113
Project Name:	OSU Aquatic Toxicology Lab	Report Revision No.: 0
Type:	QC	
Matrix:	Water	

LCS ID	Analyte	Spike Amount	Sample Result	Units	% Recovery	Analysis Method	Date Analyzed
General Chemistry							
BS1W0119	BOD5	198	198	mg/L	100	SM5210B	01/19/17

U=Not detected and reported as less than detection limit
J=Estimated value below reporting limit
E=Estimated value above calibration range
*=See case narrative

CH2MHILL Applied Sciences Laboratory
CHAIN OF CUSTODY RECORD
AND AGREEMENT TO PERFORM SERVICES

Chain of Custody Record

1100 NE Circle Blvd. Suite 300
Corvallis, OR 97330
(541) 768-3120

Client Contact

Project Name: OSU_January 2017
Project # or PO #: —
Company Name: OSU Aquatic Tox Lab
Address: 33972 Texas St.SW
City/State/Zip: Albany, OR 97321
Project Manager: Allison Cardwell
Phone #: 541-926-1254
Report to email: allison.cardwell@oregonstate.edu

Analysis Turnaround Time

TAT is Calander days
TAT if different from below ______
☒ 21 days (STD)
☐ 14 days * ☐ 3 day *
☐ 7 days * ☐ 2 days *
☐ 5 days * ☐ 1 day *
* (Surcharges will apply)

For Lab Use Only:

SDG: R1113
Custody Seals Intact? ☐ Yes ☒ No
Hand delivered? ☒ Yes ☐ No
Cooler Temp 5.9 °C
Therm ID No. 173 Therm Exp. 4/17/17
Packing Material: Circle Below
Ice [✓ Blue Ice] Box Bubble Wrap
Radiological Screen? ☐ Yes ☒ No

Sample Identification (Limit of 20 characters)	Sample Date	Sample Time	Sample Type (C=Comp, G=Grab)	Matrix (Water, Soil, Air)	Total # of Cont.	Ca, Mg, Na, K	Cl^-, SO_4	DOC	Priority Pollutants (see list below)*	Iron (Fe) by ICP-OES	BOD 5-day				Sample Specific Notes:	Lab ID:
Preservation Used						4	1	1	4	4	1					
Ni 1132R #1 new	1/12/17	1000	G	H_2O	3	X	X	X								1
Ni 1132R #8 new	1/12/17	1005	G	H_2O	1			X								2
Ni 1132R #1 old	1/13/17	1100	G	H_2O	1			X								3
Ni 1132R #8 old	1/13/17	1105	G	H_2O	1			X								4
Paulina_LM1/2	1/18/17	1200	G	H_2O	3	X	X	X								5
Paulina_1 filt	1/19/17	0900	G	H_2O	3	X	X	X								6
Paulina_1 filt-PP	1/19/17	1200	G	H_2O	1				X	X						7
OSU Effluent	1/19/17	1215	G	H_2O	1						X					8

Preservation Used: 1= Ice, 2= HCl; 3= H2SO4; 4=HNO3; 5=NaOH; 6= Other ______

Possible Hazard Identification:
Are samples hazardous? ☐ Yes ☒ No
If yes, select hazard(s): ☐ Listed ☐ Ignitable ☐ Corrosive ☐ Reactive ☐ Toxic
If YES or NO is not checked above, samples will be assumed hazardous and hazardous disposal fees will be applied.

Sample Disposal (A fee may be added if samples are retained longer than 30 day per client request, samples are returned to client, or classified as hazardous.)
☐ Return to Client ☒ Disposal by Lab ☐ Archive for ______ months

Sampled By: Allison Cardwell Allison Cardwell Date/Time: 1/19/17 @ 1330
Relinquished by: Emily Stef Emily Stefansson Date/Time: 1/19/17 @ 1420
Received by: Date/Time:
Relinquished by: Date/Time:
Received in Laboratory by: Date/Time: 1/19/17 1420
Shipped Via: ☐ UPS ☐ Fed-Ex ☐ USPS ☐ Other Tracking #:

Special Instructions/QC Requirements
*Priority Pollutants: Ag, Al, Se, Be, As, Cd, Cr, Cu, Pb, Ni, Zn, Sb (EPA 200.8)
** If any samples are outside of standard holding time/temperature → proceed with analysis (as per client)

CH2M Applied Sciences Laboratory

Sample Receipt Record

SDG ID: R1113

Date Received: 1/19/2017

Client/Project: OSU Aqua Tox

Received by: PC

Were custody seals intact and on the outside of the cooler?	☐ Yes	☐ No	☑ N/A
Shipping Record:	☑ Hand Delivered	☐ On File	☐ COC
Radiological Screening for DoD	☐ Yes	☐ No	☑ N/A
Packing Material: ☑ Hand Delivered	☐ Ice	☐ Blue Ice	☐ Box
Temp OK? (<6C) Therm ID: TH173 Exp. 4/17/17 5.9°C	☑ Yes	☐ No	☐ N/A
Was a Chain of Custody (CoC) Provided?	☑ Yes	☐ No	☐ N/A
Was the CoC correctly filled out (If No, document below)	☑ Yes	☐ No	☐ N/A
Did sample labels agree with COC? (If No, document below)	☑ Yes	☐ No	☐ N/A
Did the CoC list a correct bottle count and the preservative types (No=Correct on CoC)	☑ Yes	☐ No	☐ N/A
Were the sample containers in good condition (not broken or leaking)?	☑ Yes	☐ No	☐ N/A
Was enough sample volume provided for analysis? (If No, document below)	☑ Yes	☐ No	☐ N/A
Containers supplied by ASL?	☑ Yes	☐ No	☐ N/A
Any sample with < 1/2 holding time remaining? If so contact LPM and document below.	☐ Yes	☑ No	☐ N/A
Samples have multi-phase? If yes, document on SRER	☐ Yes	☑ No	☐ N/A
All water VOCs free of air bubbles? No, document on SRER	☐ Yes	☐ No	☑ N/A
pH of all samples met criteria on receipt? If "No", preserve and document below.	☑ Yes	☐ No	☐ N/A
Dissolved/Soluble metals filtered in the field?	☐ Yes	☐ No	☑ N/A
Dissolved/Soluble metals have sediment in bottom of container? If so document below.	☐ Yes	☐ No	☑ N/A

Preservation Adjustment

Sample ID	Reagent	Reagent Lot Number	Volume Added	Initials/Date-Time	24 hour pH check Initials/Time

Did pH of all metals samples preserved upon receipt meet criteria 24 hours after preservation? ☐ Yes ☐ No

Sample Exception Report (The following exceptions were noted)

1. Aliquots for DOC analysis filtered into 8oz polys with 1mL H2SO4 (lot#1023) using Whatman 0.45um filters (lot#G9968159). Samples Ni 1132R #8 new and Ni 1132R #8 old (R1113-02, 04) filtered using lot# A10136839.

Client was notified on:

Client contact:

Resolution to Exception:

APPENDIX C

Metals Data Summaries

Nickelous Chloride, 6-Hydrate, Crystal

`BAKER ANALYZED´® Reagent
(nickel(II) chloride, hexahydrate)

Product No. 2768
Lot No. L05582
Release Date 02/09/2012

Certificate of Analysis

TEST	SPECIFICATION	RESULT
Assay ($NiCl_2 \cdot 6H_2O$) (by EDTA titrn)	97.0 - 103.0 %	100.0 %
Insoluble Matter	0.005 % max.	0.004 %
pH of 5% Solution at 25°C	4.0 - 7.0	6.1
Sulfate (SO_4)	0.005 % max.	< 0.005 %
Nitrogen Compounds (as N)	0.005 % max.	0.005 %
Barium (Ba)	0.005 % max.	< 0.0005 %
Calcium (Ca)	0.005 % max.	< 0.0005 %
Iron (Fe)	0.002 % max.	< 0.0003 %
Lead (Pb)	0.001 % max.	< 0.0005 %
Magnesium (Mg)	0.005 % max.	< 0.0005 %
Potassium (K)	0.001 % max.	0.0002 %
Sodium (Na)	0.01 % max.	< 0.001 %
Cobalt (Co)	0.002 % max.	< 0.0002 %
Trace Impurities (in ppm):		
Copper (Cu)	5 max.	< 1
Lithium (Li)	1 max.	< 0.5
Zinc (Zn)	50 max.	< 5

Country of Origin: INDIA

ISO
Phillipsburg, NJ 9001.2008, 14001.2004
Paris, KY 9001.2008
Mexico City, Mexico 9001.2008
Deventer, The Netherlands 9001.2008, 14001.2004, 13485.2003
Selangor, Malaysia 9001.2008
Panoli, India 9001.2008
Gliwice, Poland 9001.2008, 17025.2005

Richard M. Siberski
Global Director of Quality Assurance

For questions on this Certificate of Analysis please contact Technical Services at 855-282-6867 or 610-573-2600

Avantor ™ Performance Materials. Inc.
3477 Corporate Parkway • Suite #200 • Center Valley, PA 18034 • U.S.A. • Phone: 610.573.2600 • Fax: 610.573.2610

Project: Water Effect Ratio (WER) Testing: 7-day *Ceriodaphnia dubia* chronic
Study Sponsor: Sanitary District of Decatur
Testing Facility: Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox)
"New" = Samples taken immediately before use in testing (prior to initiation or water renewal, following 3-hr equilibrium period)
"Old" = Samples taken from a composite of all replicates within a treatment following transfer of test organisms

Test #: Ni WER 1126 CDC
Test Description: Nickel Spiked Simulated Effluent/Laboratory Water (20% diluted) with DOC
Test Dates: 12/16/16 - 12/23/16
Control/Dilution water: 20% Diluted Simulated Effluent/Lab Water with DOC

	TOTAL CONC. µg/L Ni					NEW WATERS		OLD WATERS		ALL TOTAL	
Nominal Conc. µg/L Ni	**Day 0 New**	**Day 3 New**	**Day 4 Old**	**Day 6 New**	**Day 7 Old**	**Average Total Conc. µg/L Ni**	**Std Dev Total Conc. µg/L Ni**	**Average Total Conc. µg/L Ni**	**Std Dev Total Conc. µg/L Ni**	**Average Total Conc. µg/L Ni**	**Std Dev Total Conc. µg/L Ni**
	12/16/2016	12/19/2016	12/20/2016	12/22/2016	12/23/2016						
0 (Control)	1.6	1.5	1.5	1.6	1.5	1.6	0.1	1.5	0.0	1.5	0.1
4.5	6.0	4.9	5.0	5.1	5.3	5.3	0.6	5.2	0.2	5.3	0.4
6.5	6.4	6.4	6.6	6.6	7.3	6.5	0.1	7.0	0.5	6.7	0.4
9.2	8.5	8.6	8.5	10.4	8.8	9.2	1.1	8.7	0.2	9.0	0.8
13.2	12.2	11.8	11.8	12.2	11.9	12.1	0.2	11.9	0.1	12.0	0.2
18.9	16.2	16.3	17.1	17.8	17.6	16.8	0.9	17.4	0.4	17.0	0.7
26.9	22.2	22.7	23.6	23.5	23.2	22.8	0.7	23.4	0.3	23.0	0.6
38.5	32.6	31.9	32.1	34.6	32.0	33.0	1.4	32.1	0.1	32.6	1.1

Method Blanks were not analyzed due to error in sample custody. The method blank samples were part of the concurrent non-DOC test which was re-tested and therefore the original samples for that test were not analyzed.

ASC 1/6/17
ES 3/27/17

Project: Water Effect Ratio (WER) Testing: 7-day *Ceriodaphnia dubia* chronic
Study Sponsor: Sanitary District of Decatur
Testing Facility: Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox)
"New" = Samples taken immediately before use in testing (prior to initiation or water renewal, following 3-hr equilibrium period)
"Old" = Samples taken from a composite of all replicates within a treatment following transfer of test organisms

Test #: Ni WER 1126 CDC
Test Description: Nickel Spiked Simulated Effluent/Laboratory Water (20% diluted) with DOC
Test Dates: 12/16/16 - 12/23/16
Control/Dilution water: 20% Diluted Simulated Effluent/Lab Water with DOC

	DISSOLVED CONC. µg/L Ni					NEW WATERS		OLD WATERS		ALL DISSOLVED	
Nominal Conc. µg/L Ni	Day 0 New	Day 3 New	Day 4 Old	Day 6 New	Day 7 Old	Average Dissolved Conc. µg/L Ni	Std Dev Dissolved Conc. µg/L Ni	Average Dissolved Conc. µg/L Ni	Std Dev Dissolved Conc. µg/L Ni	Average Dissolved Conc. µg/L Ni	Std Dev Dissolved Conc. µg/L Ni
	12/16/2016	12/19/2016	12/20/2016	12/22/2016	12/23/2016						
0 (Control)	1.6	1.6	1.6	1.7	1.5	1.6	0.1	1.6	0.1	1.6	0.1
4.5	5.7	5.2	5.1	5.8	5.0	5.6	0.3	5.1	0.1	5.4	0.4
6.5	7.0	6.8	6.8	7.0	6.5	6.9	0.1	6.7	0.2	6.8	0.2
9.2	9.2	9.1	8.9	9.2	8.4	9.2	0.1	8.7	0.4	9.0	0.3
13.2	13.3	12.3	12.2	12.1	11.5	12.6	0.6	11.9	0.5	12.3	0.6
18.9	17.4	17.0	17.5	18.0	17.0	17.5	0.5	17.3	0.4	17.4	0.4
26.9	24.6	23.7	23.8	23.2	23.0	23.8	0.7	23.4	0.6	23.7	0.6
38.5	33.3	33.1	32.5	32.9	30.9	33.1	0.2	31.7	1.1	32.5	1.0

Method Blanks were not analyzed due to error in sample custody. The method blank samples were part of the concurrent non-DOC test which was re-tested and therefore the original samples for that test were not analyzed.

ASC 1/6/17
ES 3/27/17

Testing Performed by:	OSU Aquatic Toxicology Laboratory
Analytical Performed by:	J. Muratli /W.M. Keck Collaboratory for Plasma Spectrometry
Test Number	Ni Wer 1126 CDC
Test Dates	12/16/16 – 12/23/16
Test Description	
Test Concentration Series (µg/L Ni)	Control (0), 4.5, 6.5, 9.2, 13.2, 18.9, 26.9, 38.5.
Analytical Technique	ICPMS (Thermo X-Series II) 01/05/17; Method Detection Limit 0.016 ppb Ni

Sample ID	Metal Phase (Total or Diss.)	Nominal Concentration (ug/L Ni)	Measured [Ni] (ug/L)	Uncertainty	Day	Type	Dilution Factor	Sample Date
Ni WER 1126 CDC 6684T	T	Control (0)	1.6	0.1	0	New	1	12/16/16
Ni WER 1126 CDC 6685T	T	4.5	6.0	0.1	0	New	1	
Ni WER 1126 CDC 6686T	T	6.5	6.4	0.1	0	New	1	
Ni WER 1126 CDC 6687T	T	9.2	8.5	0.1	0	New	1	
Ni WER 1126 CDC 6688T	T	13.2	12.2	0.1	0	New	1	
Ni WER 1126 CDC 6689T	T	18.9	16.2	0.2	0	New	1	
Ni WER 1126 CDC 6690T	T	26.9	22.2	0.1	0	New	1	
Ni WER 1126 CDC 6691T	T	38.5	32.6	0.2	0	New	1	
Ni WER 1126 CDC 11011D	D	Control (0)	1.6	0.1	0	New	1	12/16/16
Ni WER 1126 CDC 11012D	D	4.5	5.7	0.1	0	New	1	
Ni WER 1126 CDC 11013D	D	6.5	7.0	0.1	0	New	1	
Ni WER 1126 CDC 11014D	D	9.2	9.2	0.1	0	New	1	
Ni WER 1126 CDC 11015D	D	13.2	13.3	0.1	0	New	1	
Ni WER 1126 CDC 11016D	D	18.9	17.4	0.2	0	New	1	
Ni WER 1126 CDC 11017D	D	26.9	24.6	0.3	0	New	1	
Ni WER 1126 CDC 11018D	D	38.5	33.3	0.2	0	New	1	
Ni WER 1126 CDC 6717T	T	Control (0)	1.5	0.1	3	New	1	12/19/16
Ni WER 1126 CDC 6718T	T	4.5	4.9	0.1	3	New	1	
Ni WER 1126 CDC 6719T	T	6.5	6.4	0.1	3	New	1	
Ni WER 1126 CDC 6720T	T	9.2	8.6	0.1	3	New	1	
Ni WER 1126 CDC 6721T	T	13.2	11.8	0.1	3	New	1	
Ni WER 1126 CDC 6722T	T	18.9	16.3	0.2	3	New	1	
Ni WER 1126 CDC 6723T	T	26.9	22.7	0.1	3	New	1	
Ni WER 1126 CDC 6724T	T	38.5	31.9	0.3	3	New	1	
Ni WER 1126 CDC 11044D	D	Control (0)	1.6	0.1	3	New	1	12/19/16
Ni WER 1126 CDC 11045D	D	4.5	5.2	0.1	3	New	1	
Ni WER 1126 CDC 11046D	D	6.5	6.8	0.1	3	New	1	
Ni WER 1126 CDC 11047D	D	9.2	9.1	0.1	3	New	1	
Ni WER 1126 CDC 11048D	D	13.2	12.3	0.1	3	New	1	
Ni WER 1126 CDC 11049D	D	18.9	17.0	0.2	3	New	1	
Ni WER 1126 CDC 11050D	D	26.9	23.7	0.1	3	New	1	
Ni WER 1126 CDC 11051D	D	38.5	33.1	0.3	3	New	1	
Ni WER 1126 CDC 6735T	T	Control (0)	1.5	0.1	4	Old	1	12/20/16
Ni WER 1126 CDC 6736T	T	4.5	5.0	0.1	4	Old	1	
Ni WER 1126 CDC 6737T	T	6.5	6.6	0.1	4	Old	1	
Ni WER 1126 CDC 6738T	T	9.2	8.5	0.1	4	Old	1	
Ni WER 1126 CDC 6739T	T	13.2	11.8	0.1	4	Old	1	
Ni WER 1126 CDC 6740T	T	18.9	17.1	0.1	4	Old	1	
Ni WER 1126 CDC 6741T	T	26.9	23.6	0.2	4	Old	1	
Ni WER 1126 CDC 6742T	T	38.5	32.1	0.2	4	Old	1	
Ni WER 1126 CDC 11062D	D	Control (0)	1.6	0.1	4	Old	1	12/20/16
Ni WER 1126 CDC 11063D	D	4.5	5.1	0.1	4	Old	1	
Ni WER 1126 CDC 11064D	D	6.5	6.8	0.1	4	Old	1	
Ni WER 1126 CDC 11065D	D	9.2	8.9	0.1	4	Old	1	
Ni WER 1126 CDC 11066D	D	13.2	12.2	0.2	4	Old	1	
Ni WER 1126 CDC 11067D	D	18.9	17.5	0.1	4	Old	1	
Ni WER 1126 CDC 11068D	D	26.9	23.8	0.1	4	Old	1	
Ni WER 1126 CDC 11069D	D	38.5	32.5	0.1	4	Old	1	

Analyst:

Date: 1/6/17

ASC 1/6/17

Sample ID	Metal Phase (Total or Diss.)	Nominal Concentration (ug/L Ni)	Measured [Ni] (ug/L)	Uncertainty	Day	Type	Dilution Factor	Sample Date
Ni WER 1126 CDC 6753T	T	Control (0)	1.6	0.1	6	New	1	12/22/16
Ni WER 1126 CDC 6754T	T	4.5	5.1	0.1	6	New	1	
Ni WER 1126 CDC 6755T	T	6.5	6.6	0.1	6	New	1	
Ni WER 1126 CDC 6756T	T	9.2	10.4	0.1	6	New	1	
Ni WER 1126 CDC 6757T	T	13.2	12.2	0.1	6	New	1	
Ni WER 1126 CDC 6758T	T	18.9	17.8	0.1	6	New	1	
Ni WER 1126 CDC 6759T	T	26.9	23.5	0.1	6	New	1	
Ni WER 1126 CDC 6760T	T	38.5	34.6	1.8	6	New	5	
Ni WER 1126 CDC 11080D	D	Control (0)	1.7	0.1	6	New	1	12/22/16
Ni WER 1126 CDC 11081D	D	4.5	5.8	0.1	6	New	1	
Ni WER 1126 CDC 11082D	D	6.5	7.0	0.1	6	New	1	
Ni WER 1126 CDC 11083D	D	9.2	9.2	0.1	6	New	1	
Ni WER 1126 CDC 11084D	D	13.2	12.1	0.1	6	New	1	
Ni WER 1126 CDC 11085D	D	18.9	18.0	0.4	6	New	1	
Ni WER 1126 CDC 11086D	D	26.9	23.2	0.1	6	New	1	
Ni WER 1126 CDC 11087D	D	38.5	32.9	0.2	6	New	1	
Ni WER 1126 CDC 6788T	T	Control (0)	1.5	0.1	7	Old	1	12/23/16
Ni WER 1126 CDC 6789T	T	4.5	5.3	0.1	7	Old	1	
Ni WER 1126 CDC 6790T	T	6.5	7.3	0.1	7	Old	1	
Ni WER 1126 CDC 6791T	T	9.2	8.8	0.1	7	Old	1	
Ni WER 1126 CDC 6792T	T	13.2	11.9	0.1	7	Old	1	
Ni WER 1126 CDC 6793T	T	18.9	17.6	0.2	7	Old	1	
Ni WER 1126 CDC 6794T	T	26.9	23.2	0.3	7	Old	1	
Ni WER 1126 CDC 6795T	T	38.5	32.0	0.1	7	Old	1	
Ni WER 1126 CDC 11114D	D	Control (0)	1.5	0.1	7	Old	1	12/23/16
Ni WER 1126 CDC 11115D	D	4.5	5.0	0.1	7	Old	1	
Ni WER 1126 CDC 11116D	D	6.5	6.5	0.1	7	Old	1	
Ni WER 1126 CDC 11117D	D	9.2	8.4	0.1	7	Old	1	
Ni WER 1126 CDC 11118D	D	13.2	11.5	0.1	7	Old	1	
Ni WER 1126 CDC 11119D	D	18.9	17.0	0.1	7	Old	1	
Ni WER 1126 CDC 11120D	D	26.9	23.0	0.2	7	Old	1	
Ni WER 1126 CDC 11121D	D	38.5	30.9	0.2	7	Old	1	

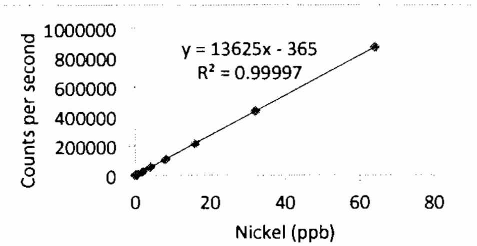
Analyst:

Date: 1/6/17

ASC 1/6/17

Date of Run	Linear Dynamic Range (LDR)	$\Sigma(x_i)^2$	$\Sigma(x_i)$	D	n	Sample Reps	Detection Limit (ppb)
1/5/17	64	5.46E+03	127	1.31E+05	27	3.0	0.016
		x	y				Limit of Quantization
	Standard Uncertainty	4.4%	0.7%				0.018

1% HNO3	Slope	Intercept	
Slope	13625	-365	Intercept
Stdev	31	799	Stdev
R^2	0.99997	1800	Stdev (Y)



Calibration Average	[Ni] (ppb)	Uncertainty	Average	Stdev	Rstdev	Blank Corrected Avg.
Standard 0	0	0.0	0	24		0
Standard 1	0.5	0.0	6926	70	1.01%	6926
Standard 3	2	0.1	27506	162	0.59%	27506
Standard 4	4	0.2	55680	134	0.24%	55680
Standard 5	8	0.4	107461	1501	1.40%	107461
Standard 6	16	0.8	214096	1850	0.86%	214096
Standard 7	32	1.6	437179	3187	0.73%	437179
Standard 8	64	3.2	871734	2869	0.33%	871734

Do not use Standard 2 for calibration, In counts are too low.

Standard Addition Added (ppb): 10

Average % Accuracy 2.7%
Average Standard % Recovery 105.5%
Average Sample % Recovery 99.2%

Average Precision 2.1%

			Instrument Data					**Calculated Raw Data**				Corrected For Dilution						
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Standard 0	0	24	0%	100.00%	1.18%	0.00	0.00	0	0.0	0.1	0	BMDL	0.0	0.1		
1		Standard 1	6926	70	1%	94.96%	0.46%	0.51	0.01	6926	0.5	0.1	0.5	0.5	0.5	0.1	7.0%	107.0%
1		Standard 2	15374	313	2%	*32.54%*	39.91%	1.13	0.02	15374	1.2	0.1	1	1.1	1.2	0.1	15.5%	115.5%
1		Standard 3	27506	162	1%	93.96%	2.11%	2.02	0.01	27506	2.0	0.1	2	2.0	2.0	0.1	2.3%	102.3%
1		Standard 4	55680	134	0%	96.91%	0.51%	4.09	0.01	55680	4.1	0.1	4	4.1	4.1	0.1	2.8%	102.8%
1		Standard 5	107461	1501	1%	95.91%	0.74%	7.89	0.11	107461	7.9	0.1	8	7.9	7.9	0.1	1.1%	98.9%
1		Standard 6	214096	1850	1%	102.03%	0.71%	15.72	0.14	214096	15.7	0.2	16	15.7	15.7	0.2	1.6%	98.4%
1		Standard 7	437179	3187	1%	98.41%	0.41%	32.11	0.23	437179	32.1	0.3	32	32.1	32.1	0.3	0.4%	100.4%
1		Standard 8	871734	2869	0%	99.28%	0.33%	64.02	0.21	871734	64.0	0.3	64	64.0	64.0	0.3	0.0%	100.0%
1		Memory Blank	629	765	122%	97.72%	0.44%	0.05	0.06	629	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	137616	866	1%	96.84%	0.57%	10.11	0.06	137616	10.1	0.1	10	10.1	10.1	0.1	1.3%	101.3%
1		Ni WER 1126 CDC T 6684	22056	147	1%	85.95%	0.97%	1.62	0.01	22056	1.6	0.1	Control (0)	1.6	1.6	0.1		
1		Ni WER 1126 CDC T 6685	81249	245	0%	89.49%	0.32%	5.97	0.02	81249	6.0	0.1	4.5	6.0	6.0	0.1		
1		Ni WER 1126 CDC T 6686	86729	410	0%	88.31%	1.55%	6.37	0.03	86729	6.4	0.1	6.5	6.4	6.4	0.1		
1		Ni WER 1126 CDC T 6687	115575	400	0%	89.59%	0.97%	8.49	0.03	115575	8.5	0.1	9.2	8.5	8.5	0.1		
1		Ni WER 1126 CDC T 6688	165982	1407	1%	87.27%	0.50%	12.19	0.10	165982	12.2	0.1	13.2	12.2	12.2	0.1		
1		Ni WER 1126 CDC T 6689	219725	2851	1%	87.97%	1.18%	16.14	0.21	219725	16.2	0.2	18.9	16.1	16.2	0.2		
1		Ni WER 1126 CDC T 6690	302595	1633	1%	90.15%	0.61%	22.22	0.12	302595	22.2	0.1	26.9	22.2	22.2	0.1		
1		Ni WER 1126 CDC T 6691	443857	3038	1%	90.95%	0.75%	32.60	0.22	443857	32.6	0.2	38.5	32.6	32.6	0.2		
1		Ni WER 1126 CDC T 6717	19415	245	1%	90.25%	0.95%	1.43	0.02	19415	1.5	0.1	Control (0)	1.4	1.5	0.1		
1		Ni WER 1126 CDC T 6718	66705	140	0%	92.67%	0.78%	4.90	0.01	66705	4.9	0.1	4.5	4.9	4.9	0.1		
1		Blank	530	22	4%	109.59%	0.13%	0.04	0.00	530	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	143395	606	0%	104.86%	0.54%	10.53	0.04	143395	10.6	0.1	10	10.5	10.6	0.1	5.5%	105.5%
1		Ni WER 1126 CDC T 6687	121128	519	0%	87.59%	0.32%	8.90	0.04	121128	8.9	0.1	9.2	8.9	8.9	0.1	2.3%	102.3%
1		Ni WER 1126 CDC T 6688 +SA	299066	1388	0%	89.14%	0.68%	21.96	0.10	299066	22.0	0.1	23.2	22.0	22.0	0.1	-2.3%	97.7%
1		Ni WER 1126 CDC T 6719	86368	1092	1%	91.11%	0.30%	6.34	0.08	86368	6.4	0.1	6.5	6.3	6.4	0.1		
1		Ni WER 1126 CDC T 6720	116825	925	1%	90.96%	0.45%	8.58	0.07	116825	8.6	0.1	9.2	8.6	8.6	0.1		
1		Ni WER 1126 CDC T 6721	160557	766	0%	89.45%	0.44%	11.79	0.06	160557	11.8	0.1	13.2	11.8	11.8	0.1		
1		Ni WER 1126 CDC T 6722	221676	1857	1%	89.59%	0.88%	16.28	0.14	221676	16.3	0.2	18.9	16.3	16.3	0.2		
1		Ni WER 1126 CDC T 6723	309309	857	0%	90.29%	0.38%	22.72	0.06	309309	22.7	0.1	26.9	22.7	22.7	0.1		
1		Ni WER 1126 CDC T 6724	434394	3488	1%	89.98%	1.59%	31.90	0.26	434394	31.9	0.3	38.5	31.9	31.9	0.3		
1		Ni WER 1126 CDC T 6735	20054	311	2%	87.07%	0.15%	1.47	0.02	20054	1.5	0.1	Control (0)	1.5	1.5	0.1		
1		Ni WER 1126 CDC T 6736	67353	662	1%	91.11%	1.31%	4.95	0.05	67353	5.0	0.1	4.5	4.9	5.0	0.1		
1		Ni WER 1126 CDC T 6737	90003	686	1%	90.55%	0.78%	6.61	0.05	90003	6.6	0.1	6.5	6.6	6.6	0.1		
1		Ni WER 1126 CDC T 6738	115791	301	0%	92.06%	0.34%	8.50	0.02	115791	8.5	0.1	9.2	8.5	8.5	0.1		
1		Blank	538	10	2%	108.28%	1.87%	0.04	0.00	538	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	143592	1674	1%	107.28%	1.74%	10.55	0.12	143592	10.6	0.1	10	10.6	10.6	0.1	5.7%	105.7%
1		Ni WER 1126 CDC T 6724	432986	1933	0%	90.69%	0.03%	31.80	0.14	432986	31.8	0.2	38.5	31.8	31.8	0.2	0.2%	99.8%
1		Ni WER 1126 CDC T 6736 +5A	200382	1265	1%	88.95%	0.64%	14.72	0.09	200382	14.7	0.1	14.5	14.7	14.7	0.1	-2.4%	97.6%
1		Ni WER 1126 CDC T 6739	160197	60	0%	90.42%	0.35%	11.77	0.00	160197	11.8	0.1	13.2	11.8	11.8	0.1		
1		Ni WER 1126 CDC T 6740	232243	511	0%	89.56%	0.22%	17.06	0.04	232243	17.1	0.1	18.9	17.1	17.1	0.1		
1		Ni WER 1126 CDC T 6741	321165	2839	1%	88.70%	0.86%	23.59	0.21	321165	23.6	0.2	26.9	23.6	23.6	0.2		
1		Ni WER 1126 CDC T 6742	437539	1930	0%	93.51%	0.45%	32.13	0.14	437539	32.1	0.2	38.5	32.1	32.1	0.2		
1		Ni WER 1126 CDC T 6753	21437	569	3%	92.58%	1.31%	1.57	0.04	21437	1.6	0.1	Control (0)	1.6	1.6	0.1		
1		Ni WER 1126 CDC T 6754	69440	592	1%	93.47%	0.27%	5.10	0.04	69440	5.1	0.1	4.5	5.1	5.1	0.1		
1		Ni WER 1126 CDC T 6755	89102	329	0%	95.00%	0.36%	6.54	0.02	89102	6.6	0.1	6.5	6.5	6.6	0.1		
1		Ni WER 1126 CDC T 6756	140771	1296	1%	93.41%	0.35%	10.34	0.10	140771	10.4	0.1	9.2	10.3	10.4	0.1		

1/6/17

ASC 1/6/17

					Instrument Data				Calculated Raw Data				Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Ni WER 1126 CDC T 6757	166410	1315	1%	88.85%	0.17%	12.22	0.10	166410	12.2	0.1	13.2	12.2	12.2	0.1		
1		Ni WER 1126 CDC T 6758	242818	807	0%	89.52%	0.83%	17.83	0.06	242818	17.8	0.1	18.9	17.8	17.8	0.1		
1		Blank	521	27	5%	103.13%	0.17%	0.04	0.00	521	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	146461	1740	1%	107.52%	0.75%	10.76	0.13	146461	10.8	0.2	10	10.8	10.8	0.2	7.8%	107.8%
1		Ni WER 1126 CDC T 6741	320332	1312	0%	90.42%	0.23%	23.53	0.10	320332	23.5	0.1	26.9	23.5	23.5	0.1	0.1%	99.9%
1		Ni WER 1126 CDC T 6742 +SA	569230	4784	1%	91.58%	0.55%	41.80	0.35	569230	41.8	0.4	48.5	41.8	41.8	0.4	-3.3%	96.7%
1		Ni WER 1126 CDC T 6759	320346	1619	1%	90.21%	0.24%	23.53	0.12	320346	23.5	0.1	26.9	23.5	23.5	0.1		
1		Ni WER 1126 CDC T 6760	552716	11673	2%	*72.13%*	1.40%	40.59	0.86	552716	40.6	0.9	38.5	40.6	40.6	0.9	In counts low – rerun	
1		Ni WER 1126 CDC T 6788	20449	167	1%	90.76%	0.27%	1.50	0.01	20449	1.5	0.1	Control (0)	1.5	1.5	0.1		
1		Ni WER 1126 CDC T 6789	71925	286	0%	86.82%	0.60%	5.28	0.02	71925	5.3	0.1	4.5	5.3	5.3	0.1		
1		Ni WER 1126 CDC T 6790	98758	343	0%	86.88%	0.18%	7.25	0.03	98758	7.3	0.1	6.5	7.3	7.3	0.1		
1		Ni WER 1126 CDC T 6791	119877	383	0%	89.61%	0.09%	8.80	0.03	119877	8.8	0.1	9.2	8.8	8.8	0.1		
1		Ni WER 1126 CDC T 6792	161353	745	0%	90.17%	0.23%	11.85	0.06	161353	11.9	0.1	13.2	11.9	11.9	0.1		
1		Ni WER 1126 CDC T 6793	239294	2638	1%	87.62%	1.00%	17.57	0.19	239294	17.6	0.2	18.9	17.6	17.6	0.2		
1		Ni WER 1126 CDC T 6794	316291	3649	1%	95.22%	1.68%	23.23	0.27	316291	23.2	0.3	26.9	23.2	23.2	0.3		
1		Ni WER 1126 CDC T 6795	435188	1082	0%	99.36%	1.26%	31.96	0.08	435188	32.0	0.1	38.5	32.0	32.0	0.1		
1		Blank	644	20	3%	110.44%	2.36%	0.05	0.00	644	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	145216	1282	1%	117.13%	2.74%	10.66	0.09	145216	10.7	0.1	10	10.7	10.7	0.1	6.9%	106.9%
1		Ni WER 1126 CDC T 6789	71842	484	1%	92.25%	1.13%	5.28	0.04	71842	5.3	0.1	4.5	5.3	5.3	0.1	0.1%	99.9%
1		Ni WER 1126 CDC T 6790 +SA	227228	1228	1%	91.17%	0.22%	16.69	0.09	227228	16.7	0.1	16.5	16.7	16.7	0.1	-5.7%	94.3%
1		Ni WER 1126 CDC D 11011	22010	260	1%	88.16%	1.08%	1.62	0.02	22010	1.6	0.1	Control (0)	1.6	1.6	0.1		
1		Ni WER 1126 CDC D 11012	76909	1037	1%	88.86%	1.04%	5.65	0.08	76909	5.7	0.1	4.5	5.6	5.7	0.1		
1		Ni WER 1126 CDC D 11013	95156	990	1%	88.13%	0.77%	6.99	0.07	95156	7.0	0.1	6.5	7.0	7.0	0.1		
1		Ni WER 1126 CDC D 11014	125021	1405	1%	89.46%	0.83%	9.18	0.10	125021	9.2	0.1	9.2	9.2	9.2	0.1		
1		Ni WER 1126 CDC D 11015	180946	1430	1%	88.21%	0.37%	13.29	0.11	180946	13.3	0.1	13.2	13.3	13.3	0.1		
1		Ni WER 1126 CDC D 11016	236717	2916	1%	87.54%	0.74%	17.38	0.21	236717	17.4	0.2	18.9	17.4	17.4	0.2		
1		Ni WER 1126 CDC D 11017	334491	4105	1%	87.27%	0.53%	24.57	0.30	334491	24.6	0.3	26.9	24.6	24.6	0.3		
1		Ni WER 1126 CDC D 11018	453225	1974	0%	88.56%	0.30%	33.29	0.15	453225	33.3	0.2	38.5	33.3	33.3	0.2		
1		Ni WER 1126 CDC D 11044	21753	233	1%	89.28%	0.73%	1.60	0.02	21753	1.6	0.1	Control (0)	1.6	1.6	0.1		
1		Ni WER 1126 CDC D 11045	70890	728	1%	90.06%	0.40%	5.21	0.05	70890	5.2	0.1	4.5	5.2	5.2	0.1		
1		Blank	388	30	8%	99.66%	1.55%	0.03	0.00	388	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	149945	1582	1%	106.21%	1.14%	11.01	0.12	149945	11.0	0.1	10	11.0	11.0	0.1	10.3%	110.3%
1		Ni WER 1126 CDC D 11012	76723	637	1%	85.93%	1.28%	5.64	0.05	76723	5.7	0.1	4.5	5.6	5.7	0.1	0.1%	99.9%
1		Ni WER 1126 CDC D 11013 +SA	238970	2277	1%	84.50%	0.41%	17.55	0.17	238970	17.6	0.2	16.5	17.6	17.6	0.2	5.6%	105.6%
1		Ni WER 1126 CDC D 11046	92110	213	0%	91.04%	0.33%	6.77	0.02	92110	6.8	0.1	6.5	6.8	6.8	0.1		
1		Ni WER 1126 CDC D 11047	123143	825	1%	87.33%	0.29%	9.04	0.06	123143	9.1	0.1	9.2	9.0	9.1	0.1		
1		Ni WER 1126 CDC D 11048	166911	688	0%	89.63%	0.54%	12.26	0.05	166911	12.3	0.1	13.2	12.3	12.3	0.1		
1		Ni WER 1126 CDC D 11049	230960	2124	1%	89.21%	1.20%	16.96	0.16	230960	17.0	0.2	18.9	17.0	17.0	0.2		
1		Ni WER 1126 CDC D 11050	322712	480	0%	86.35%	0.63%	23.70	0.04	322712	23.7	0.1	26.9	23.7	23.7	0.1		
1		Ni WER 1126 CDC D 11051	450896	3486	1%	90.99%	0.17%	33.11	0.26	450896	33.1	0.3	38.5	33.1	33.1	0.3		
1		Ni WER 1126 CDC D 11062	21548	368	2%	94.75%	0.70%	1.58	0.03	21548	1.6	0.1	Control (0)	1.6	1.6	0.1		
1		Ni WER 1126 CDC D 11063	68659	453	1%	94.75%	0.62%	5.04	0.03	68659	5.1	0.1	4.5	5.0	5.1	0.1		
1		Ni WER 1126 CDC D 11064	92084	661	1%	87.66%	0.34%	6.76	0.05	92084	6.8	0.1	6.5	6.8	6.8	0.1		
1		Ni WER 1126 CDC D 11065	120535	966	1%	88.47%	0.72%	8.85	0.07	120535	8.9	0.1	9.2	8.9	8.9	0.1		
1		Blank	412	31	8%	99.18%	0.96%	0.03	0.00	412	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	153368	1115	1%	100.10%	0.67%	11.26	0.08	153368	11.3	0.1	10	11.3	11.3	0.1	12.8%	112.8%
1		Ni WER 1126 CDC D 11047	123842	944	1%	85.69%	0.91%	9.10	0.07	123842	9.1	0.1	9.2	9.1	9.1	0.1	0.3%	100.3%
1		Ni WER 1126 CDC D 11050 +SA	460633	3395	1%	89.04%	0.89%	33.83	0.25	460633	33.8	0.3	36.9	33.8	33.8	0.3	1.2%	101.2%
1		Ni WER 1126 CDC D 11066	165404	1801	1%	85.06%	0.63%	12.15	0.13	165404	12.2	0.2	13.2	12.2	12.2	0.2		
1		Ni WER 1126 CDC D 11067	237877	1124	0%	87.89%	0.25%	17.47	0.08	237877	17.5	0.1	18.9	17.5	17.5	0.1		
1		Ni WER 1126 CDC D 11068	323573	1099	0%	89.71%	0.46%	23.76	0.08	323573	23.8	0.1	26.9	23.8	23.8	0.1		
1		Ni WER 1126 CDC D 11069	442213	939	0%	91.49%	0.27%	32.48	0.07	442213	32.5	0.1	38.5	32.5	32.5	0.1		
1		Ni WER 1126 CDC D 11080	22833	210	1%	88.29%	2.72%	1.68	0.02	22833	1.7	0.1	Control (0)	1.7	1.7	0.1		
1		Ni WER 1126 CDC D 11081	78567	797	1%	83.34%	0.86%	5.77	0.06	78567	5.8	0.1	4.5	5.8	5.8	0.1		
1		Ni WER 1126 CDC D 11082	95373	573	1%	87.96%	0.45%	7.00	0.04	95373	7.0	0.1	6.5	7.0	7.0	0.1		
1		Ni WER 1126 CDC D 11083	124685	972	1%	98.73%	0.42%	9.16	0.07	124685	9.2	0.1	9.2	9.2	9.2	0.1		
1		Ni WER 1126 CDC D 11084	164148	861	1%	97.43%	0.16%	12.06	0.06	164148	12.1	0.1	13.2	12.1	12.1	0.1		
1		Ni WER 1126 CDC D 11085	245018	5262	2%	93.78%	2.22%	17.99	0.39	245018	18.0	0.4	18.9	18.0	18.0	0.4		
1		8lank	427	56	13%	113.02%	0.96%	0.03	0.00	427	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	148148	1313	1%	115.95%	0.92%	10.88	0.10	148148	10.9	0.1	10	10.9	10.9	0.1	9.0%	109.0%
1		Ni WER 1126 CDC D 11069	435937	358	0%	95.89%	0.17%	32.02	0.03	435937	32.0	0.1	38.5	32.0	32.0	0.1	0.7%	99.3%
1		Ni WER 1126 CDC D 11083 +SA	261090	778	0%	99.48%	1.78%	19.17	0.06	261090	19.2	0.1	19.2	19.2	19.2	0.1	0.1%	100.1%
1		Ni WER 1126 CDC D 11086	315538	1376	0%	95.21%	0.29%	23.17	0.10	315538	23.2	0.1	26.9	23.2	23.2	0.1		

1/6/17

ASC 1/6/17

			Instrument Data					Calculated Raw Data					Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Ni WER 1126 CDC D 11087	448539	2504	1%	97.18%	0.73%	32.94	0.18	448539	32.9	0.2	38.5	32.9	32.9	0.2		
1		Ni WER 1126 CDC D 11114	20603	312	2%	96.60%	1.03%	1.51	0.02	20603	1.5	0.1	Control (0)	1.5	1.5	0.1		
1		Ni WER 1126 CDC D 11115	67703	313	0%	96.64%	0.28%	4.97	0.02	67703	5.0	0.1	4.5	5.0	5.0	0.1		
1		Ni WER 1126 CDC D 11116	88222	590	1%	94.67%	0.70%	6.48	0.04	88222	6.5	0.1	6.5	6.5	6.5	0.1		
1		Ni WER 1126 CDC D 11117	113805	1368	1%	96.05%	0.78%	8.36	0.10	113805	8.4	0.1	9.2	8.4	8.4	0.1		
1		Ni WER 1126 CDC D 11118	156617	842	1%	96.97%	0.45%	11.50	0.06	156617	11.5	0.1	13.2	11.5	11.5	0.1		
1		Ni WER 1126 CDC D 11119	231028	461	0%	95.23%	0.58%	16.97	0.03	231028	17.0	0.1	18.9	17.0	17.0	0.1		
1		Ni WER 1126 CDC D 11120	312476	2271	1%	96.59%	1.16%	22.95	0.17	312476	23.0	0.2	26.9	23.0	23.0	0.2		
1		Ni WER 1126 CDC D 11121	420653	2897	1%	98.36%	0.67%	30.89	0.21	420653	30.9	0.2	38.5	30.9	30.9	0.2		
1		Blank	411	13	3%	115.34%	1.12%	0.03	0.00	411	0.1	0.1	0	0.0	0.1	0.1		
1		QC Standard	147406	1671	1%	*122.80%*	1.76%	10.83	0.12	147406	10.8	0.1	10	10.8	10.8	0.1	8.5%	108.5%
1		Ni WER 1126 CDC D 11115	66235	470	1%	102.95%	0.62%	4.86	0.04	66235	4.9	0.1	4.5	4.9	4.9	0.1	1.1%	98.9%
1		Ni WER 1126 CDC D 11116 +SA	216297	573	0%	108.20%	1.10%	15.89	0.04	216297	15.9	0.1	16.5	15.9	15.9	0.1	-6.0%	94.0%
1		OSU Effluent 12/08/16	11616	151	1%	107.15%	0.94%	0.85	0.01	11616	0.9	0.1	0	0.9	0.9	0.1		
1		OSU Effluent 12/22/16	2895	25	1%	101.21%	0.79%	0.21	0.00	2895	0.2	0.1	0	0.2	0.2	0.1		
1		Ni WER 1126 CDC T 6760	388919	5951	2%	99.56%	1.50%	28.56	0.44	388919	28.6	0.4	38.5	28.6	28.6	0.4	In slow to stabilize – rerun	
1		Blank	376	15	4%	92.90%	1.04%	0.03	0.00	376	0.1	0.1	0	0.0	0.1	0.1		
1		Ni WER 1126 CDC T 6760	459958	13291	3%	*79.57%*	2.95%	33.78	0.98	459958	33.8	1.0	38.5	33.8	33.8	1.0	In counts low – dilute and rerun	
5	0.25	Ni WER 1126 CDC T 6760	93967	470	1%	92.82%	0.41%	34.51	0.17	93967	6.9	0.1	38.5	34.5	34.6	1.8	DILUTED RERUN	

1/6/17

ASC 1/6/17

Project: Water Effect Ratio (WER) Testing: 7-day *Ceriodaphnia dubia* chronic
Study Sponsor: Sanitary District of Decatur
Testing Facility: Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox)
"New" = Samples taken immediately before use in testing (prior to initiation or water renewal, following 3-hr equilibrium period)
"Old" = Samples taken from a composite of all replicates within a treatment following transfer of test organisms

Test #: Ni WER 1132R CDC
Test Description: Nickel Spiked Simulated Effluent/Laboratory Water (20% diluted)
Test Dates: 1/9/17 - 1/16/17
Control/Dilution water: 20% Diluted Simulated Effluent/Lab Water (no added DOC)

	TOTAL CONC. µg/L Ni					**NEW WATERS**		**OLD WATERS**		**ALL TOTAL**	
Nominal Conc. µg/L Ni	**Day 0 New**	**Day 3 New**	**Day 4 Old**	**Day 6 New**	**Day 7 Old**	**Average Total Conc. µg/L Ni**	**Std Dev Total Conc. µg/L Ni**	**Average Total Conc. µg/L Ni**	**Std Dev Total Conc. µg/L Ni**	**Average Total Conc. µg/L Ni**	**Std Dev Total Conc. µg/L Ni**
	1/9/2017	1/12/2017	1/13/2017	1/15/2017	1/16/2017						
Method Blk	< 0.023	0.05	< 0.023	< 0.023	< 0.023						
VHW RW	1.2	1.1	1.0	Sample error	1.1	1.1	0.0	1.1	0.1	1.1	0.0
0 (Control)	1.3	1.3	1.4	1.5	1.4	1.3	0.1	1.4	0.0	1.4	0.1
2.1	2.9	3.0	2.8	3.0	2.9	3.0	0.0	2.9	0.1	2.9	0.1
2.9	3.5	3.7	4.0	3.7	3.7	3.6	0.1	3.8	0.2	3.7	0.2
4.2	4.5	4.7	4.4	4.7	4.4	4.6	0.2	4.4	0.0	4.5	0.2
6	5.9	6.4	5.8	6.5	6.1	6.3	0.3	6.0	0.2	6.1	0.3
8.5	7.9	8.4	8.1	8.3	8.1	8.2	0.3	8.1	0.0	8.1	0.2
12.2	10.5	11.4	10.6	11.4	10.8	11.1	0.5	10.7	0.1	10.9	0.4
17.4	14.4	15.5	14.9	16.3	15.5	15.4	1.0	15.2	0.4	15.3	0.7

ASC 2/10/17
ES 3/27/17

Project: Water Effect Ratio (WER) Testing: 7-day *Ceriodaphnia dubia* chronic
Study Sponsor: Sanitary District of Decatur
Testing Facility: Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox)
"New" = Samples taken immediately before use in testing (prior to initiation or water renewal, following 3-hr equilibrium period)
"Old" = Samples taken from a composite of all replicates within a treatment following transfer of test organisms

Test #: Ni WER 1132R CDC
Test Description: Nickel Spiked Simulated Effluent/Laboratory Water (20% diluted)
Test Dates: 1/9/17 - 1/16/17
Control/Dilution water: 20% Diluted Simulated Effluent/Lab Water (no added DOC)

	DISSOLVED CONC. µg/L Ni					**NEW WATERS**		**OLD WATERS**		**ALL DISSOLVED**	
Nominal Conc. µg/L Ni	**Day 0 New**	**Day 3 New**	**Day 4 Old**	**Day 6 New**	**Day 7 Old**	**Average Dissolved Conc. µg/L Ni**	**Std Dev Dissolved Conc. µg/L Ni**	**Average Dissolved Conc. µg/L Ni**	**Std Dev Dissolved Conc. µg/L Ni**	**Average Dissolved Conc. µg/L Ni**	**Std Dev Dissolved Conc. µg/L Ni**
	1/9/2017	1/12/2017	1/13/2017	1/15/2017	1/16/2017						
Method Blk	0.07	0.04	0.09	0.18	0.08						
VHW RW	1.2	1.2	1.1	1.3	1.1	1.2	0.1	1.1	0.0	1.2	0.1
0 (Control)	1.3	1.3	1.2	1.5	1.2	1.3	0.1	1.2	0.0	1.3	0.1
2.1	2.9	3.0	2.6	3.2	2.8	3.0	0.2	2.7	0.1	2.9	0.2
2.9	3.5	3.7	3.2	3.9	3.3	3.7	0.2	3.3	0.1	3.5	0.3
4.2	4.5	4.8	4.1	4.9	4.0	4.7	0.2	4.1	0.1	4.5	0.4
6	5.9	6.5	5.6	6.7	5.5	6.4	0.4	5.5	0.1	6.0	0.5
8.5	7.9	8.5	7.5	8.6	7.3	8.3	0.4	7.4	0.1	8.0	0.6
12.2	10.6	11.5	10.1	11.7	10.1	11.3	0.6	10.1	0.0	10.8	0.8
17.4	14.6	16.0	14.0	16.4	14.7	15.7	0.9	14.3	0.5	15.1	1.0

ASC 2/10/17
ES 3/27/17

Testing Performed by:	OSU Aquatic Toxicology Laboratory
Analytical Performed by:	J. Muratli /W.M. Keck Collaboratory for Plasma Spectrometry
Test Number	Ni Wer 1132R CDC
Test Dates	1/09/17 – 1/16/17
Test Description	
Test Concentration Series (µg/L Ni)	VHW Control (0), Control (0), 2.1, 2.9, 4.2, 6.0, 8.5, 12.2, 17.4
Analytical Technique	ICPMS (Thermo X-Series II) 02/07/17; Method Detection Limit 0.023 ppb Ni

Sample ID	Metal Phase (Total or Diss.)	Nominal Concentration (ug/L Ni)	Measured [Ni] (ug/L)	Uncertainty	Day	Type	Dilution Factor	Sample Date
Ni WER 1132R CDC 6823T	T	Method Blk (0)	BMDL	-	0	New	1	01/09/17
Ni WER 1132R CDC 6824T	T	VHW Ctl	1.15	0.06	0	New	1	
Ni WER 1132R CDC 6825T	T	0 (Con)	1.25	0.06	0	New	1	
Ni WER 1132R CDC 6826T	T	2.1	2.91	0.06	0	New	1	
Ni WER 1132R CDC 6827T	T	2.9	3.48	0.06	0	New	1	
Ni WER 1132R CDC 6828T	T	4.2	4.46	0.06	0	New	1	
Ni WER 1132R CDC 6829T	T	6	5.93	0.07	0	New	1	
Ni WER 1132R CDC 6830T	T	8.5	7.87	0.08	0	New	1	
Ni WER 1132R CDC 6831T	T	12.2	10.52	0.11	0	New	1	
Ni WER 1132R CDC 6832T	T	17.4	14.35	0.06	0	New	1	
Ni WER 1132R CDC 11158D	D	Method Blk (0)	0.07	0.06	0	New	1	01/09/17
Ni WER 1132R CDC 11159D	D	VHW Ctl	1.17	0.06	0	New	1	
Ni WER 1132R CDC 11160D	D	0 (Con)	1.26	0.06	0	New	1	
Ni WER 1132R CDC 11161D	D	2.1	2.85	0.06	0	New	1	
Ni WER 1132R CDC 11162D	D	2.9	3.53	0.06	0	New	1	
Ni WER 1132R CDC 11163D	D	4.2	4.52	0.06	0	New	1	
Ni WER 1132R CDC 11164D	D	6	5.88	0.06	0	New	1	
Ni WER 1132R CDC 11165D	D	8.5	7.92	0.07	0	New	1	
Ni WER 1132R CDC 11166D	D	12.2	10.64	0.09	0	New	1	
Ni WER 1132R CDC 11167D	D	17.4	14.64	0.09	0	New	1	
Ni WER 1132R CDC 6851T	T	Method Blk (0)	0.05	0.06	3	New	1	01/12/17
Ni WER 1132R CDC 6852T	T	VHW Ctl	1.10	0.06	3	New	1	
Ni WER 1132R CDC 6853T	T	0 (Con)	1.28	0.06	3	New	1	
Ni WER 1132R CDC 6854T	T	2.1	3.00	0.06	3	New	1	
Ni WER 1132R CDC 6855T	T	2.9	3.65	0.06	3	New	1	
Ni WER 1132R CDC 6856T	T	4.2	4.74	0.06	3	New	1	
Ni WER 1132R CDC 6857T	T	6	6.40	0.06	3	New	1	
Ni WER 1132R CDC 6858T	T	8.5	8.37	0.07	3	New	1	
Ni WER 1132R CDC 6859T	T	12.2	11.41	0.15	3	New	1	
Ni WER 1132R CDC 6860T	T	17.4	15.54	0.07	3	New	1	
Ni WER 1132R CDC 11186D	D	Method Blk (0)	0.04	0.06	3	New	1	01/12/17
Ni WER 1132R CDC 11187D	D	VHW Ctl	1.20	0.06	3	New	1	
Ni WER 1132R CDC 11188D	D	0 (Con)	1.31	0.06	3	New	1	
Ni WER 1132R CDC 11189D	D	2.1	3.01	0.06	3	New	1	
Ni WER 1132R CDC 11190D	D	2.9	3.67	0.06	3	New	1	
Ni WER 1132R CDC 11191D	D	4.2	4.82	0.06	3	New	1	
Ni WER 1132R CDC 11192D	D	6	6.45	0.10	3	New	1	
Ni WER 1132R CDC 11193D	D	8.5	8.48	0.06	3	New	1	
Ni WER 1132R CDC 11194D	D	12.2	11.48	0.07	3	New	1	
Ni WER 1132R CDC 11195D	D	17.4	16.02	0.09	3	New	1	
Ni WER 1132R CDC 6861T	T	Method Blk (0)	BMDL	-	4	Old	1	01/13/17
Ni WER 1132R CDC 6862T	T	VHW Ctl	1.03	0.06	4	Old	1	
Ni WER 1132R CDC 6863T	T	0 (Con)	1.44	0.06	4	Old	1	
Ni WER 1132R CDC 6864T	T	2.1	2.78	0.06	4	Old	1	
Ni WER 1132R CDC 6865T	T	2.9	4.00	0.07	4	Old	1	
Ni WER 1132R CDC 6866T	T	4.2	4.37	0.06	4	Old	1	
Ni WER 1132R CDC 6867T	T	6	5.82	0.06	4	Old	1	
Ni WER 1132R CDC 6868T	T	8.5	8.05	0.11	4	Old	1	
Ni WER 1132R CDC 6869T	T	12.2	10.63	0.06	4	Old	1	
Ni WER 1132R CDC 6870T	T	17.4	14.93	0.12	4	Old	1	
Ni WER 1132R CDC 11196D	D	Method Blk (0)	0.09	0.06	4	Old	1	01/13/17
Ni WER 1132R CDC 11197D	D	VHW Ctl	1.14	0.06	4	Old	1	
Ni WER 1132R CDC 11198D	D	0 (Con)	1.23	0.06	4	Old	1	
Ni WER 1132R CDC 11199D	D	2.1	2.63	0.06	4	Old	1	
Ni WER 1132R CDC 11200D	D	2.9	3.23	0.06	4	Old	1	
Ni WER 1132R CDC 11201D	D	4.2	4.13	0.06	4	Old	1	
Ni WER 1132R CDC 11202D	D	6	5.60	0.06	4	Old	1	
Ni WER 1132R CDC 11203D	D	8.5	7.49	0.07	4	Old	1	
Ni WER 1132R CDC 11204D	D	12.2	10.08	0.06	4	Old	1	
Ni WER 1132R CDC 11205D	D	17.4	13.98	0.14	4	Old	1	

Analyst:

Date: 2/9/17

ASC 2/10/17

Sample ID	Metal Phase (Total or Diss.)	Nominal Concentration (ug/L Ni)	Measured [Ni] (ug/L)	Uncertainty	Day	Type	Dilution Factor	Sample Date
Ni WER 1132R CDC 6871T	T	Method Blk (0)	BMDL	-	6	New	1	01/15/17
Ni WER 1132R CDC 6872T	T	VHW Ctl	7.83 *	0.09	6	New	1	
Ni WER 1132R CDC 6873T	T	0 (Con)	1.49	0.06	6	New	1	
Ni WER 1132R CDC 6874T	T	2.1	2.98	0.06	6	New	1	
Ni WER 1132R CDC 6875T	T	2.9	3.69	0.06	6	New	1	
Ni WER 1132R CDC 6876T	T	4.2	4.70	0.07	6	New	1	
Ni WER 1132R CDC 6877T	T	6	6.49	0.06	6	New	1	
Ni WER 1132R CDC 6878T	T	8.5	8.30	0.10	6	New	1	
Ni WER 1132R CDC 6879T	T	12.2	11.37	0.06	6	New	1	
Ni WER 1132R CDC 6880T	T	17.4	16.25	0.08	6	New	5	
Ni WER 1132R CDC 11206D	D	Method Blk (0)	0.18	0.06	6	New	1	01/15/17
Ni WER 1132R CDC 11207D	D	VHW Ctl	1.31	0.06	6	New	1	
Ni WER 1132R CDC 11208D	D	0 (Con)	1.45	0.06	6	New	1	
Ni WER 1132R CDC 11209D	D	2.1	3.23	0.06	6	New	1	
Ni WER 1132R CDC 11210D	D	2.9	3.90	0.06	6	New	1	
Ni WER 1132R CDC 11211D	D	4.2	4.85	0.06	6	New	1	
Ni WER 1132R CDC 11212D	D	6	6.72	0.07	6	New	1	
Ni WER 1132R CDC 11213D	D	8.5	8.57	0.06	6	New	1	
Ni WER 1132R CDC 11214D	D	12.2	11.69	0.08	6	New	1	
Ni WER 1132R CDC 11215D	D	17.4	16.35	0.09	6	New	1	
Ni WER 1132R CDC 6881T	T	Method Blk (0)	BMDL	-	7	Old	1	01/16/17
Ni WER 1132R CDC 6882T	T	VHW Ctl	1.11	0.06	7	Old	1	
Ni WER 1132R CDC 6883T	T	0 (Con)	1.37	0.06	7	Old	1	
Ni WER 1132R CDC 6884T	T	2.1	2.92	0.06	7	Old	1	
Ni WER 1132R CDC 6885T	T	2.9	3.69	0.06	7	Old	1	
Ni WER 1132R CDC 6886T	T	4.2	4.39	0.06	7	Old	1	
Ni WER 1132R CDC 6887T	T	6	6.10	0.06	7	Old	1	
Ni WER 1132R CDC 6888T	T	8.5	8.08	0.10	7	Old	1	
Ni WER 1132R CDC 6889T	T	12.2	10.77	0.07	7	Old	1	
Ni WER 1132R CDC 6890T	T	17.4	15.54	0.13	7	Old	1	
Ni WER 1132R CDC 11216D	D	Method Blk (0)	0.08	0.06	7	Old	1	01/16/17
Ni WER 1132R CDC 11217D	D	VHW Ctl	1.07	0.06	7	Old	1	
Ni WER 1132R CDC 11218D	D	0 (Con)	1.24	0.06	7	Old	1	
Ni WER 1132R CDC 11219D	D	2.1	2.79	0.06	7	Old	1	
Ni WER 1132R CDC 11220D	D	2.9	3.31	0.06	7	Old	1	
Ni WER 1132R CDC 11221D	D	4.2	3.98	0.06	7	Old	1	
Ni WER 1132R CDC 11222D	D	6	5.47	0.06	7	Old	1	
Ni WER 1132R CDC 11223D	D	8.5	7.33	0.07	7	Old	1	
Ni WER 1132R CDC 11224D	D	12.2	10.11	0.08	7	Old	1	
Ni WER 1132R CDC 11225D	D	17.4	14.65	0.16	7	Old	1	

* Mis-sampled. Remove from analysis/consideration. This sample was measured as 1.11 µg/L Ni in the old sample the next day. 2/9/17 ASC

Analyst:

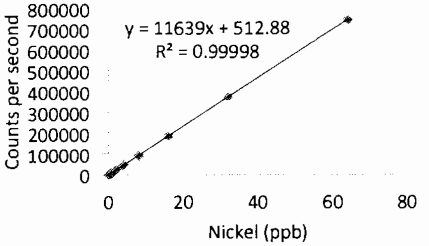
Date: 2/9/17

ASC 2/10/17

Date of Run	Linear Dynamic Range (LDR)	$\Sigma(x_i)^2$	$\Sigma(x_i)$	D	n	Sample Reps	Detection Limit (ppb)	1% HNO3	Slope	Intercept	
2/7/17	64	5.46E+03	128	1.31E+05	27	3.0	0.023	Slope	11639	513	Intercept
		x	y				Limit of Quantization	Stdev	17	428	Stdev
	Standard Uncertainty	5.0%	0.8%				0.026	R^2	0.99998	1051	Stdev (Y)

Calibration Average	[Ni] (ppb)	Uncertainty	Average	Stdev	Rstdev	Blank Corrected Avg.
Standard 0	0	0.0	0	30		0
Standard 1	0.5	0.0	6145	87	1.42%	6145
Standard 2	1	0.1	12030	140	1.16%	12030
Standard 3	2	0.1	23840	110	0.46%	23840
Standard 4	4	0.2	48612	531	1.09%	48612
Standard 5	8	0.4	92559	410	0.44%	92559
Standard 6	16	0.8	185933	825	0.44%	185933
Standard 7	32	1.6	374647	3019	0.81%	374647
Standard 8	64	3.2	744771	4294	0.58%	744771

Average Precision 1.6%



Standard Addition Added (ppb):
9

Ran the standard addition solution at the end and found it contained ~9 ppb instead of 10 ppb. Adjust the Standard Addition accordingly.

Average % Accuracy	1.9%
Average Standard % Recovery	106.1%
Average Sample % Recovery	97.1%

			Instrument Data					**Calculated Raw Data**					Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Standard 0	0	30	0%	100.00%	0.36%	0.00	0.00	0	BMDL	-	0	BMDL	BMDL	-		
1		Standard 1	6145	87	1%	87.33%	0.52%	0.53	0.01	6145	0.5	0.1	0.5	0.5	0.48	0.06	3.2%	96.8%
1		Standard 2	12030	140	1%	89.87%	0.97%	1.03	0.01	12030	1.0	0.1	1	1.0	0.99	0.06	1.0%	99.0%
1		Standard 3	23840	110	0%	97.60%	0.77%	2.05	0.01	23840	2.0	0.1	2	2.0	2.00	0.06	0.2%	100.2%
1		Standard 4	48612	531	1%	96.04%	0.59%	4.17	0.05	48612	4.1	0.1	4	4.2	4.13	0.07	3.3%	103.3%
1		Standard 5	92559	410	0%	96.56%	1.09%	7.95	0.04	92559	7.9	0.1	8	7.9	7.91	0.07	1.1%	98.9%
1		Standard 6	185933	825	0%	101.34%	0.45%	15.96	0.07	185933	15.9	0.1	16	16.0	15.93	0.09	0.4%	99.6%
1		Standard 7	374647	3019	1%	98.65%	0.49%	32.16	0.26	374647	32.1	0.3	32	32.2	32.15	0.27	0.5%	100.5%
1		Standard 8	744771	4294	1%	101.70%	0.69%	63.93	0.37	744771	63.9	0.4	64	63.9	63.95	0.38	0.1%	99.9%
1		Memory Blank	39	26	68%	96.07%	0.50%	0.00	0.00	39	BMDL	-	0	BMDL	BMDL	-		
1		QC Standard	118950	68	0%	93.85%	0.34%	10.21	0.01	118950	10.2	0.1	10	10.2	10.18	0.06	1.8%	101.8%
1		Ni WER 1132R CDC T 6823	240	38	16%	88.52%	0.64%	0.02	0.00	240	BMDL	-	Method Blk (0)	BMDL	BMDL	-		
1		Ni WER 1132R CDC T 6824	13954	94	1%	85.61%	1.78%	1.20	0.01	13954	1.2	0.1	VHW Ctl	1.2	1.15	0.06		
1		Ni WER 1132R CDC T 6825	15091	65	0%	87.43%	0.48%	1.30	0.01	15091	1.3	0.1	0 (Con)	1.3	1.25	0.06		
1		Ni WER 1132R CDC T 6826	34406	166	0%	85.66%	0.57%	2.95	0.01	34406	2.9	0.1	2.1	3.0	2.91	0.06		
1		Ni WER 1132R CDC T 6827	41055	160	0%	90.54%	0.20%	3.52	0.01	41055	3.5	0.1	2.9	3.5	3.48	0.06		
1		Ni WER 1132R CDC T 6828	52464	294	1%	92.54%	0.20%	4.50	0.03	52464	4.5	0.1	4.2	4.5	4.46	0.06		
1		Ni WER 1132R CDC T 6829	69557	446	1%	93.93%	0.37%	5.97	0.04	69557	5.9	0.1	6	6.0	5.93	0.07		
1		Ni WER 1132R CDC T 6830	92061	719	1%	94.28%	0.52%	7.90	0.06	92061	7.9	0.1	8.5	7.9	7.87	0.08		
1		Ni WER 1132R CDC T 6831	122909	1054	1%	97.52%	0.73%	10.55	0.09	122909	10.5	0.1	12.2	10.6	10.52	0.11		
1		Ni WER 1132R CDC T 6832	167539	283	0%	97.73%	0.41%	14.38	0.02	167539	14.4	0.1	17.4	14.4	14.35	0.06		
1		Blank	479	24	5%	107.11%	0.74%	0.04	0.00	479	BMDL	-	0	0.0	BMDL	-		
1		QC Standard	124429	526	0%	110.84%	0.57%	10.68	0.05	124429	10.6	0.1	10	10.7	10.65	0.07	6.5%	106.5%
1		Ni WER 1132R CDC T 6826	34406	497	1%	96.00%	0.50%	2.95	0.04	34406	2.9	0.1	2.1	3.0	2.91	0.07	0.0%	100.0%
1		Ni WER 1132R CDC T 6827 +SA	134144	894	1%	98.10%	0.95%	11.51	0.08	134144	11.5	0.1	12.9	11.5	11.48	0.09	-11.1%	88.9%
1		Ni WER 1132R CDC D 11158	1294	28	2%	113.04%	1.08%	0.11	0.00	1294	0.1	0.1	Method Blk (0)	0.1	0.07	0.06		
1		Ni WER 1132R CDC D 11159	14137	194	1%	104.24%	0.40%	1.21	0.02	14137	1.2	0.1	VHW Ctl	1.2	1.17	0.06		
1		Ni WER 1132R CDC D 11160	15194	182	1%	100.14%	0.22%	1.30	0.02	15194	1.3	0.1	0 (Con)	1.3	1.26	0.06		
1		Ni WER 1132R CDC D 11161	33714	350	1%	100.48%	0.40%	2.89	0.03	33714	2.9	0.1	2.1	2.9	2.85	0.06		
1		Ni WER 1132R CDC D 11162	41599	305	1%	99.42%	0.39%	3.57	0.03	41599	3.5	0.1	2.9	3.6	3.53	0.06		
1		Ni WER 1132R CDC D 11163	53070	298	1%	98.07%	0.62%	4.56	0.03	53070	4.5	0.1	4.2	4.6	4.52	0.06		
1		Ni WER 1132R CDC D 11164	68930	263	0%	100.41%	0.59%	5.92	0.02	68930	5.9	0.1	6	5.9	5.88	0.06		
1		Ni WER 1132R CDC D 11165	92708	423	0%	100.78%	0.41%	7.96	0.04	92708	7.9	0.1	8.5	8.0	7.92	0.07		
1		Ni WER 1132R CDC D 11166	124312	793	1%	103.74%	0.66%	10.67	0.07	124312	10.6	0.1	12.2	10.7	10.64	0.09		
1		Ni WER 1132R CDC D 11167	170850	763	0%	102.01%	0.04%	14.66	0.07	170850	14.6	0.1	17.4	14.7	14.64	0.09		
1		Blank	1638	15	1%	118.98%	0.23%	0.14	0.00	1638	0.1	0.1	0	0.1	0.10	0.06		
1		QC Standard	124474	415	0%	119.41%	0.42%	10.68	0.04	124474	10.7	0.1	10	10.7	10.65	0.07	6.5%	106.5%
1		Ni WER 1132R CDC D 11163	53003	238	0%	101.51%	0.54%	4.55	0.02	53003	4.5	0.1	4.2	4.5	4.51	0.06	0.1%	99.9%
1		Ni WER 1132R CDC D 11164 +S,	164073	496	0%	102.19%	0.96%	14.08	0.04	164073	14.1	0.1	16	14.1	14.05	0.07	-9.2%	90.8%
1		Ni WER 1132R CDC T 6851	1088	16	2%	117.22%	0.33%	0.09	0.00	1088	0.0	0.1	Method Blk (0)	0.1	0.05	0.06		
1		Ni WER 1132R CDC T 6852	13312	230	2%	106.09%	1.56%	1.14	0.02	13312	1.1	0.1	VHW Ctl	1.1	1.10	0.06		
1		Ni WER 1132R CDC T 6853	15444	354	2%	103.48%	1.26%	1.33	0.03	15444	1.3	0.1	0 (Con)	1.3	1.28	0.06		
1		Ni WER 1132R CDC T 6854	35404	229	1%	105.60%	0.75%	3.04	0.02	35404	3.0	0.1	2.1	3.0	3.00	0.06		
1		Ni WER 1132R CDC T 6855	43039	291	1%	107.44%	0.41%	3.69	0.03	43039	3.7	0.1	2.9	3.7	3.65	0.06		

Because counts of In are increasing through the run, rerun a selection of these samples later.

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JM 2/9/17

ASC 2/10/17

			Instrument Data					Calculated Raw Data					Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Ni WER 1132R CDC T 6856	55738	269	0%	107.62%	0.44%	4.78	0.02	55738	4.7	0.1	4.2	4.8	4.74	0.06		
1		Ni WER 1132R CDC T 6857	75004	304	0%	106.12%	1.00%	6.44	0.03	75004	6.4	0.1	6	6.4	6.40	0.06		
1		Ni WER 1132R CDC T 6858	97975	530	1%	107.70%	0.74%	8.41	0.05	97975	8.4	0.1	8.5	8.4	8.37	0.07		
1		Ni WER 1132R CDC T 6859	133344	1631	1%	109.63%	0.63%	11.45	0.14	133344	11.4	0.2	12.2	11.5	11.41	0.15		
1		Ni WER 1132R CDC T 6860	181390	410	0%	110.14%	0.16%	15.57	0.04	181390	15.5	0.1	17.4	15.6	15.54	0.07		
1		Blank	587	50	9%	*125.89%*	1.19%	0.05	0.00	587	BMDL	-	0	0.1	BMDL	-		
1		QC Standard	128869	586	0%	*125.05%*	0.29%	11.06	0.05	128869	11.0	0.1	10	11.1	11.03	0.07	10.3%	110.3%
1		Ni WER 1132R CDC T 6857	74532	234	0%	104.56%	0.28%	6.40	0.02	74532	6.4	0.1	6	6.4	6.36	0.06	0.3%	99.7%
1		Ni WER 1132R CDC T 6858 +5A	195013	218	0%	110.84%	0.69%	16.74	0.02	195013	16.7	0.1	18.5	16.7	16.71	0.06	-7.4%	92.6%
1		Ni WER 1132R CDC D 11186	1012	68	7%	119.93%	0.29%	0.09	0.01	1012	0.0	0.1	Method Blk (0)	0.1	0.04	0.06		
1		Ni WER 1132R CDC D 11187	14500	178	1%	105.82%	0.26%	1.25	0.02	14500	1.2	0.1	VHW Ctl	1.2	1.20	0.06		
1		Ni WER 1132R CDC D 11188	15747	83	1%	106.79%	0.55%	1.35	0.01	15747	1.3	0.1	0 (Con)	1.4	1.31	0.06		
1		Ni WER 1132R CDC D 11189	35546	70	0%	108.39%	0.11%	3.05	0.01	35546	3.0	0.1	2.1	3.1	3.01	0.06		
1		Ni WER 1132R CDC D 11190	43172	40	0%	110.80%	0.45%	3.71	0.00	43172	3.7	0.1	2.9	3.7	3.67	0.06		
1		Ni WER 1132R CDC D 11191	56666	228	0%	110.64%	0.67%	4.86	0.02	56666	4.8	0.1	4.2	4.9	4.82	0.06		
1		Ni WER 1132R CDC D 11192	75524	968	1%	109.72%	0.14%	6.48	0.08	75524	6.4	0.1	6	6.5	6.45	0.10		
1		Ni WER 1132R CDC D 11193	99215	69	0%	111.97%	0.18%	8.52	0.01	99215	8.5	0.1	8.5	8.5	8.48	0.06		
1		Ni WER 1132R CDC D 11194	134087	509	0%	112.25%	1.09%	11.51	0.04	134087	11.5	0.1	12.2	11.5	11.48	0.07		
1		Ni WER 1132R CDC D 11195	186985	840	0%	109.27%	0.99%	16.05	0.07	186985	16.0	0.1	17.4	16.1	16.02	0.09		
1		Blank	530	37	7%	119.75%	0.30%	0.05	0.00	530	BMDL	-	0	0.0	BMDL	-		
1		QC Standard	130929	347	0%	*126.25%*	0.46%	11.24	0.03	130929	11.2	0.1	10	11.2	11.21	0.06	12.1%	112.1%
1		Ni WER 1132R CDC D 11189	35702	353	1%	110.08%	0.75%	3.06	0.03	35702	3.0	0.1	2.1	3.1	3.02	0.06	0.2%	100.2%
1		Ni WER 1132R CDC D 11190 +S,	141800	747	1%	112.68%	0.42%	12.17	0.06	141800	12.1	0.1	12.9	12.2	12.14	0.08	-5.8%	94.2%
1		Ni WER 1132R CDC T 6861	748	29	4%	117.18%	1.91%	0.06	0.00	748	BMDL	-	Method Blk (0)	0.1	BMDL	-		
1		Ni WER 1132R CDC T 6862	12453	21	0%	118.15%	0.84%	1.07	0.00	12453	1.0	0.1	VHW Ctl	1.1	1.03	0.06		
1		Ni WER 1132R CDC T 6863	17304	127	1%	110.16%	0.77%	1.49	0.01	17304	1.4	0.1	0 (Con)	1.5	1.44	0.06		
1		Ni WER 1132R CDC T 6864	32860	350	1%	112.66%	0.52%	2.82	0.03	32860	2.8	0.1	2.1	2.8	2.78	0.06		
1		Ni WER 1132R CDC T 6865	47125	419	1%	114.09%	0.27%	4.05	0.04	47125	4.0	0.1	2.9	4.0	4.00	0.07		
1		Ni WER 1132R CDC T 6866	51353	401	1%	114.14%	0.13%	4.41	0.03	51353	4.4	0.1	4.2	4.4	4.37	0.06		
1		Ni WER 1132R CDC T 6867	68274	228	0%	114.75%	0.23%	5.86	0.02	68274	5.8	0.1	6	5.9	5.82	0.06		
1		Ni WER 1132R CDC T 6868	94251	1084	1%	103.35%	1.03%	8.09	0.09	94251	8.1	0.1	8.5	8.1	8.05	0.11		
1		Ni WER 1132R CDC T 6869	124238	379	0%	115.06%	0.47%	10.66	0.03	124238	10.6	0.1	12.2	10.7	10.63	0.06		
1		Ni WER 1132R CDC T 6870	174257	1256	1%	115.20%	0.50%	14.96	0.11	174257	14.9	0.1	17.4	15.0	14.93	0.12		
1		Blank	459	35	8%	*133.05%*	0.52%	0.04	0.00	459	BMDL	-	0	0.0	BMDL	-		
1		QC Standard	131548	837	1%	*130.99%*	1.03%	11.29	0.07	131548	11.3	0.1	10	11.3	11.26	0.09	12.6%	112.6%
1		Ni WER 1132R CDC T 6865	46523	53	0%	113.72%	0.52%	3.99	0.01	46523	4.0	0.1	2.9	4.0	3.95	0.06	0.6%	99.4%
1		Ni WER 1132R CDC T 6866 +SA	149981	716	0%	113.49%	0.53%	12.87	0.06	149981	12.8	0.1	14.2	12.9	12.84	0.08	-5.8%	94.2%
1		Ni WER 1132R CDC D 11196	1585	46	3%	*128.01%*	0.81%	0.14	0.00	1585	0.1	0.1	Method Blk (0)	0.1	0.09	0.06		
1		Ni WER 1132R CDC D 11197	13819	68	0%	*121.83%*	1.16%	1.19	0.01	13819	1.1	0.1	VHW Ctl	1.2	1.14	0.06		
1		Ni WER 1132R CDC D 11198	14777	83	1%	115.16%	0.98%	1.27	0.01	14777	1.2	0.1	0 (Con)	1.3	1.23	0.06		
1		Ni WER 1132R CDC D 11199	31117	398	1%	115.34%	0.86%	2.67	0.03	31117	2.6	0.1	2.1	2.7	2.63	0.06		
1		Ni WER 1132R CDC D 11200	38147	296	1%	117.24%	0.55%	3.27	0.03	38147	3.2	0.1	2.9	3.3	3.23	0.06		
1		Ni WER 1132R CDC D 11201	48628	151	0%	116.66%	1.07%	4.17	0.01	48628	4.1	0.1	4.2	4.2	4.13	0.06		
1		Ni WER 1132R CDC D 11202	65690	271	0%	117.99%	0.42%	5.64	0.02	65690	5.6	0.1	6	5.6	5.60	0.06		
1		Ni WER 1132R CDC D 11203	87716	436	0%	116.00%	0.66%	7.53	0.04	87716	7.5	0.1	8.5	7.5	7.49	0.07		
1		Ni WER 1132R CDC D 11204	117783	159	0%	118.74%	0.32%	10.11	0.01	117783	10.1	0.1	12.2	10.1	10.08	0.06		
1		Ni WER 1132R CDC D 11205	163245	1528	1%	118.97%	0.38%	14.01	0.13	163245	14.0	0.1	17.4	14.0	13.98	0.14		
1		Blank	450	24	5%	*135.59%*	0.76%	0.04	0.00	450	BMDL	-	0	0.0	BMDL	-		
1		QC Standard	131084	951	1%	*135.62%*	0.62%	11.25	0.08	131084	11.2	0.1	10	11.3	11.22	0.10	12.2%	112.2%
1		Ni WER 1132R CDC D 11201	48459	192	0%	116.27%	0.56%	4.16	0.02	48459	4.1	0.1	4.2	4.2	4.12	0.06	0.2%	99.8%
1		Ni WER 1132R CDC D 11202 +S,	165388	276	0%	117.26%	1.48%	14.20	0.02	165388	14.2	0.1	16	14.2	14.17	0.06	-4.8%	95.2%
1		Ni WER 1132R CDC T 6871	594	24	4%	*126.92%*	0.84%	0.05	0.00	594	BMDL	-	Method Blk (0)	0.1	BMDL	-		
1		Ni WER 1132R CDC T 6872	91636	785	1%	*128.26%*	0.17%	7.87	0.07	91636	7.8	0.1	VHW Ctl	7.9	7.83	0.09	Rerun this one– high conc	
1		Ni WER 1132R CDC T 6873	17909	155	1%	117.40%	1.34%	1.54	0.01	17909	1.5	0.1	0 (Con)	1.5	1.49	0.06		
1		Ni WER 1132R CDC T 6874	35233	183	1%	*122.83%*	0.81%	3.02	0.02	35233	3.0	0.1	2.1	3.0	2.98	0.06		
1		Ni WER 1132R CDC T 6875	43448	270	1%	*121.21%*	0.57%	3.73	0.02	43448	3.7	0.1	2.9	3.7	3.69	0.06		
1		Ni WER 1132R CDC T 6876	55169	427	1%	*120.12%*	0.40%	4.74	0.04	55169	4.7	0.1	4.2	4.7	4.70	0.07		
1		Ni WER 1132R CDC T 6877	76067	283	0%	119.24%	0.55%	6.53	0.02	76067	6.5	0.1	6	6.5	6.49	0.06		
1		Ni WER 1132R CDC T 6878	97111	937	1%	*121.07%*	1.03%	8.34	0.08	97111	8.3	0.1	8.5	8.3	8.30	0.10		
1		Ni WER 1132R CDC T 6879	132887	147	0%	*120.94%*	0.09%	11.41	0.01	132887	11.4	0.1	12.2	11.4	11.37	0.06		
1		Ni WER 1132R CDC T 6880	189632	711	0%	116.68%	0.51%	16.28	0.06	189632	16.2	0.1	17.4	16.3	16.25	0.08		
1		Blank	474	21	5%	*137.21%*	1.01%	0.04	0.00	474	BMDL	-	0	0.0	BMDL	-		
1		QC Standard	130865	684	1%	*137.19%*	0.92%	11.23	0.06	130865	11.2	0.1	10	11.2	11.20	0.08	12.0%	112.0%

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JW 2/9/17

ASC 2/10/17

				Instrument Data					Calculated Raw Data				Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Ni WER 1132R CDC T 6874	36571	478	1%	114.61%	2.02%	3.14	0.04	36571	3.1	0.1	2.1	3.1	3.10	0.07	1.9%	101.9%
1		Ni WER 1132R CDC T 6875 +SA	149009	729	0%	110.64%	0.64%	12.79	0.06	149009	12.8	0.1	12.9	12.8	12.76	0.08	0.8%	100.8%
1		Ni WER 1132R CDC D 11206	2622	210	8%	*132.13%*	1.38%	0.23	0.02	2622	0.2	0.1	Method Blk (0)	0.2	0.18	0.06		
1		Ni WER 1132R CDC D 11207	15704	325	2%	*122.68%*	1.37%	1.35	0.03	15704	1.3	0.1	VHW Ctl	1.3	1.31	0.06		
1		Ni WER 1132R CDC D 11208	17405	223	1%	*122.39%*	0.80%	1.49	0.02	17405	1.5	0.1	0 (Con)	1.5	1.45	0.06		
1		Ni WER 1132R CDC D 11209	38061	94	0%	117.32%	1.90%	3.27	0.01	38061	3.2	0.1	2.1	3.3	3.23	0.06		
1		Ni WER 1132R CDC D 11210	45890	118	0%	119.51%	0.37%	3.94	0.01	45890	3.9	0.1	2.9	3.9	3.90	0.06		
1		Ni WER 1132R CDC D 11211	56936	37	0%	*121.97%*	0.18%	4.89	0.00	56936	4.8	0.1	4.2	4.9	4.85	0.06		
1		Ni WER 1132R CDC D 11212	78717	456	1%	118.71%	0.41%	6.76	0.04	78717	6.7	0.1	6	6.8	6.72	0.07		
1		Ni WER 1132R CDC D 11213	100288	328	0%	119.60%	0.40%	8.61	0.03	100288	8.6	0.1	8.5	8.6	8.57	0.06		
1		Ni WER 1132R CDC D 11214	136607	628	0%	*121.02%*	0.77%	11.73	0.05	136607	11.7	0.1	12.2	11.7	11.69	0.08		
1		Ni WER 1132R CDC D 11215	190799	801	0%	*125.47%*	0.40%	16.38	0.07	190799	16.3	0.1	17.4	16.4	16.35	0.09		
1		Blank	467	28	6%	*144.69%*	1.40%	0.04	0.00	467	BMDL	-	0	0.0	BMDL	-		
1		QC Standard	133047	646	0%	*140.28%*	0.73%	11.42	0.06	133047	11.4	0.1	10	11.4	11.39	0.08	13.9%	113.9%
1		Ni WER 1132R CDC D 11210	45465	369	1%	119.29%	1.04%	3.90	0.03	45465	3.9	0.1	2.9	3.9	3.86	0.06	0.5%	99.5%
1		Ni WER 1132R CDC D 11211 +5,	158329	1286	1%	*123.17%*	1.41%	13.59	0.11	158329	13.6	0.1	14.2	13.6	13.56	0.12	-3.2%	96.8%
1		Ni WER 1132R CDC T 6881	507	8	2%	*134.78%*	0.42%	0.04	0.00	507	BMDL	-	Method Blk (0)	0.0	BMDL	-		
1		Ni WER 1132R CDC T 6882	13393	82	1%	116.20%	1.11%	1.15	0.01	13393	1.1	0.1	VHW Ctl	1.2	1.11	0.06		
1		Ni WER 1132R CDC T 6883	16501	165	1%	118.51%	0.69%	1.42	0.01	16501	1.4	0.1	0 (Con)	1.4	1.37	0.06		
1		Ni WER 1132R CDC T 6884	34504	263	1%	*122.64%*	0.50%	2.96	0.02	34504	2.9	0.1	2.1	3.0	2.92	0.06		
1		Ni WER 1132R CDC T 6885	43463	99	0%	*120.62%*	0.57%	3.73	0.01	43463	3.7	0.1	2.9	3.7	3.69	0.06		
1		Ni WER 1132R CDC T 6886	51626	236	0%	*120.91%*	0.58%	4.43	0.02	51626	4.4	0.1	4.2	4.4	4.39	0.06		
1		Ni WER 1132R CDC T 6887	71502	288	0%	116.27%	1.08%	6.14	0.03	71502	6.1	0.1	6	6.1	6.10	0.06		
1		Ni WER 1132R CDC T 6888	94590	958	1%	119.52%	0.22%	8.12	0.08	94590	8.1	0.1	8.5	8.1	8.08	0.10		
1		Ni WER 1132R CDC T 6889	125822	578	0%	*121.68%*	0.73%	10.80	0.05	125822	10.8	0.1	12.2	10.8	10.77	0.07		
1		Ni WER 1132R CDC T 6890	181429	1347	1%	119.87%	0.75%	15.57	0.12	181429	15.5	0.1	17.4	15.6	15.54	0.13		
1		Blank	724	19	3%	*141.20%*	0.25%	0.06	0.00	724	BMDL	-	0	0.1	BMDL	-		
1		QC Standard	133506	691	1%	*139.13%*	0.68%	11.46	0.06	133506	11.4	0.1	10	11.5	11.43	0.08	14.3%	114.3%
1		Ni WER 1132R CDC T 6886	50784	251	0%	*120.81%*	1.02%	4.36	0.02	50784	4.3	0.1	4.2	4.4	4.32	0.06	0.8%	99.2%
1		Ni WER 1132R CDC T 6887 +5A	170354	1072	1%	*122.51%*	0.86%	14.62	0.09	170354	14.6	0.1	16	14.6	14.59	0.11	-5.6%	94.4%
1		Ni WER 1132R CDC D 11216	1481	11	1%	*134.52%*	1.61%	0.13	0.00	1481	0.1	0.1	Method Blk (0)	0.1	0.08	0.06		
1		Ni WER 1132R CDC D 11217	12993	83	1%	*127.43%*	0.17%	1.12	0.01	12993	1.1	0.1	VHW Ctl	1.1	1.07	0.06		
1		Ni WER 1132R CDC D 11218	14960	145	1%	*125.71%*	0.23%	1.28	0.01	14960	1.2	0.1	0 (Con)	1.3	1.24	0.06		
1		Ni WER 1132R CDC D 11219	32957	78	0%	*122.92%*	0.31%	2.83	0.01	32957	2.8	0.1	2.1	2.8	2.79	0.06		
1		Ni WER 1132R CDC D 11220	39020	224	1%	*121.94%*	0.07%	3.35	0.02	39020	3.3	0.1	2.9	3.3	3.31	0.06		
1		Ni WER 1132R CDC D 11221	46892	259	1%	*122.54%*	0.41%	4.03	0.02	46892	4.0	0.1	4.2	4.0	3.98	0.06		
1		Ni WER 1132R CDC D 11222	64149	177	0%	*126.69%*	0.29%	5.51	0.02	64149	5.5	0.1	6	5.5	5.47	0.06		
1		Ni WER 1132R CDC D 11223	85782	469	1%	*124.10%*	0.65%	7.36	0.04	85782	7.3	0.1	8.5	7.4	7.33	0.07		
1		Ni WER 1132R CDC D 11224	118163	631	1%	*123.46%*	1.37%	10.14	0.05	118163	10.1	0.1	12.2	10.1	10.11	0.08		
1		Ni WER 1132R CDC D 11225	170982	1736	1%	*125.77%*	0.95%	14.68	0.15	170982	14.6	0.2	17.4	14.7	14.65	0.16		
1		Blank	693	42	6%	*148.31%*	0.92%	0.06	0.00	693	BMDL	-	0	0.1	BMDL	-		
1		QC Standard	134542	1439	1%	*142.07%*	0.86%	11.55	0.12	134542	11.5	0.1	10	11.6	11.52	0.14	15.2%	115.2%
1		Ni WER 1132R CDC D 11219	32668	115	0%	*125.02%*	0.55%	2.80	0.01	32668	2.8	0.1	2.1	2.8	2.76	0.06	0.4%	99.6%
1		Ni WER 1132R CDC D 11220 +S,	138251	814	1%	*126.37%*	0.46%	11.87	0.07	138251	11.8	0.1	12.9	11.9	11.83	0.09	-5.3%	94.7%
100	5	020617_Ni_100x	106753	1567	1%	*148.94%*	1.01%	916.30	13.45	106753	9.1	0.1	1000	916.3	913	48		
50	2.5	020617_Ni_50x	213056	2369	1%	*144.84%*	1.17%	914.40	10.17	213056	18.3	0.2	1000	914.4	913	47		
25	1.25	020617_Ni_25x	423548	1734	0%	*144.10%*	0.58%	908.90	3.72	423548	36.3	0.2	1000	908.9	909	46		
1		Ni WER 1132R CDC T 6872	86046	645	1%	*137.07%*	1.12%	7.39	0.06	86046	7.3	0.1	VHW Ctl	7.4	7.35	0.08		

2/9/17

ASC 2/10/17

Testing Performed by:	OSU Aquatic Toxicology Laboratory
Analytical Performed by:	J. Muratli /W.M. Keck Collaboratory for Plasma Spectrometry
Test Number	Ni Wer 1132R CDC
Test Dates	1/09/17 – 1/16/17
Test Description	
Test Concentration Series (µg/L Ni)	VHW Control (0), Control (0), 2.1, 2.9, 4.2, 6.0, 8.5, 12.2, 17.4
Analytical Technique	ICPMS (Thermo X-Series II) 02/07/17; Method Detection Limit 0.029 ppb Ni

Because the internal standard (In) counts drifted upwards through the course of the initial run of Ni WER 1132R CDC samples, I revisited a handful of samples during the effluent run as a check on the concentrations with a new standard curve. These are these results. -JMM

Sample ID	**Metal Phase (Total or Diss.)**	**Nominal Concentration (ug/L Ni)**	**Measured [Ni] (ug/L)**	**Uncertainty**	**Day**	**Type**	**Dilution Factor**	**Sample Date**
Ni WER 1132R CDC 6826T	T	2.1	2.80	0.11	0	New	1	01/09/17
Ni WER 1132R CDC 6829T	T	6	5.65	0.10	0	New	1	
Ni WER 1132R CDC 6831T	T	12.2	10.53	0.11	0	New	1	
Ni WER 1132R CDC 11161D	D	2.1	2.81	0.10	0	New	1	01/09/17
Ni WER 1132R CDC 11164D	D	6	5.88	0.10	0	New	1	
Ni WER 1132R CDC 11166D	D	12.2	10.92	0.11	0	New	1	
Ni WER 1132R CDC 11167D	D	17.4	15.06	0.13	0	New	1	
Ni WER 1132R CDC 6866T	T	4.2	4.32	0.10	4	Old	1	01/13/17
Ni WER 1132R CDC 6868T	T	8.5	7.83	0.10	4	Old	1	
Ni WER 1132R CDC 6870T	T	17.4	15.08	0.13	4	Old	1	
Ni WER 1132R CDC 11201D	D	4.2	4.17	0.10	4	Old	1	01/13/17
Ni WER 1132R CDC 11203D	D	8.5	7.54	0.10	4	Old	1	
Ni WER 1132R CDC 11205D	D	17.4	14.37	0.13	4	Old	1	

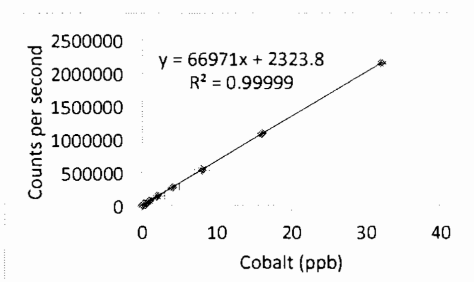
Analyst:

Date: 2/9/17

ASC 2/10/17

Date of Run	Linear Dynamic Range (LDR)	$\Sigma(x_i)^2$	$\Sigma(x_i)$	D	n	Sample Reps	Detection Limit (ppb)
2/7/17	32	1.37E+03	64	3.28E+04	27	3.0	0.008
		x	y			Limit of Quantization	
	Standard Uncertainty	5.0%	0.8%				0.005

1% HNO3	**Slope**	**Intercept**	
Slope	66971	2324	Intercept
Stdev	75	920	Stdev
R^2	0.99999	2258	Stdev (Y)



Calibration Average	[Co] (ppb)	Uncertainty	Average	Stdev	Rstdev	Blank Corrected Avg.
Standard 0	0	0.0	0	33		0
Standard 1	0.2	0.0	13583	166	1.22%	13583
Standard 2	0.5	0.0	34511	332	0.96%	34511
Standard 3	1	0.1	69772	462	0.66%	69772
Standard 4	2	0.1	140229	1510	1.08%	140229
Standard 5	4	0.2	270109	2868	1.06%	270109
Standard 6	8	0.4	538729	971	0.18%	538729
Standard 7	16	0.8	1076178	5301	0.49%	1076178
Standard 8	32	1.6	2143871	11296	0.53%	2143871

Standard Addition Added (ppb):
0

Average % Accuracy 3.4%
Average Standard % Recovery 99.4%
Average Sample % Recovery 104.0%

Average Precision 5.8%

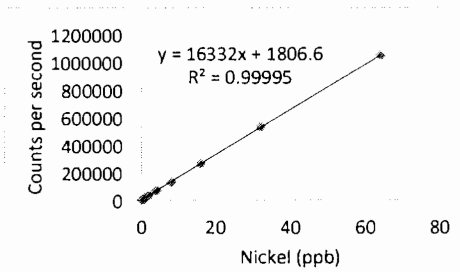
			Instrument Data					**Calculated Raw Data**					Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Co)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Co] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Co] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Co] ug/L	Calculated [Co] (ug/L)	Uncertainty	% Error	% Recovery
1		Standard 0	0	33	0%	100.00%	0.25%	0.00	0.00	0	BMDL	-	0	BMDL	BMDL	-		
1		Standard 1	13583	166	1%	90.97%	0.67%	0.20	0.00	13583	0.2	0.0	0.2	0.2	0.17	0.02	15.9%	84.1%
1		Standard 2	34511	332	1%	96.05%	0.47%	0.51	0.01	34511	0.5	0.0	0.5	0.5	0.48	0.02	3.9%	96.1%
1		Standard 3	69772	462	1%	96.93%	0.36%	1.02	0.01	69772	1.0	0.0	1	1.0	1.01	0.02	0.7%	100.7%
1		Standard 4	140229	1510	1%	94.02%	0.97%	2.06	0.02	140229	2.1	0.0	2	2.1	2.06	0.03	3.0%	103.0%
1		Standard 5	270109	2868	1%	98.91%	0.69%	3.96	0.04	270109	4.0	0.0	4	4.0	4.00	0.05	0.0%	100.0%
1		Standard 6	538729	971	0%	99.53%	0.30%	7.91	0.01	538729	8.0	0.0	8	7.9	8.01	0.03	0.1%	100.1%
1		Standard 7	1076178	5301	0%	97.59%	1.09%	15.79	0.08	1076178	16.0	0.1	16	15.8	16.03	0.08	0.2%	100.2%
1		Standard 8	2143871	11296	1%	99.57%	0.66%	31.46	0.17	2143871	32.0	0.2	32	31.5	31.98	0.17	0.1%	99.9%
1		Memory 8lank	127	56	44%	93.83%	0.11%	0.00	0.00	127	BMDL	-	0	BMDL	BMDL	-		
1		QC Standard	672770	5549	1%	91.77%	0.79%	9.87	0.08	672770	10.0	0.1	10	9.9	10.01	0.09	0.1%	100.1%
1		OSU Effluent 01/05/17 D	1656	54	3%	92.94%	0.31%	0.02	0.00	1656	BMDL	-		0.0	BMDL	-		
1		OSU Effluent 01/12/17 D	2222	88	4%	*77.70%*	0.21%	0.03	0.00	2222	BMDL	-		0.0	BMDL	-		
1		OSU Effluent 02/02/17 D	15058	63	0%	86.34%	0.86%	0.22	0.00	15058	0.2	0.0		0.2	0.19	0.02		
1		Ni WER 1132R CDC T 6826	72022	866	1%	*70.64%*	1.19%	1.06	0.01	72022	1.0	0.0		1.1	1.04	0.02		
1		Ni WER 1132R CDC T 6829	70435	425	1%	*78.91%*	0.56%	1.03	0.01	70435	1.0	0.0		1.0	1.02	0.02		
1		Ni WER 1132R CDC T 6831	72164	105	0%	*79.13%*	0.38%	1.06	0.00	72164	1.0	0.0		1.1	1.04	0.02		
1		Ni WER 1132R CDC D 11161	71751	356	0%	82.10%	0.58%	1.05	0.01	71751	1.0	0.0		1.1	1.04	0.02		
1		Ni WER 1132R CDC D 11164	73955	195	0%	83.51%	0.84%	1.09	0.00	73955	1.1	0.0		1.1	1.07	0.02		
1		Ni WER 1132R CDC D 11166	74349	569	1%	83.46%	0.13%	1.09	0.01	74349	1.1	0.0		1.1	1.08	0.02		
1		Ni WER 1132R CDC D 11167	74626	400	1%	84.50%	0.29%	1.10	0.01	74626	1.1	0.0		1.1	1.08	0.02		
1		Blank	-63	61	96%	99.25%	0.90%	0.00	0.00	-63	BMDL	-		BMDL	BMDL	-		
1		QC Standard	737893	6023	1%	95.44%	0.47%	10.83	0.09	737893	11.0	0.1	10	10.8	10.98	0.09	9.8%	109.8%
1		Ni WER 1132R CDC T 6826	77858	575	1%	*79.22%*	0.64%	1.14	0.01	77858	1.1	0.0		1.1	1.13	0.02	4.0%	104.0%
1		Ni WER 1132R CDC D 11164 +S,	74111	234	0%	84.04%	0.56%	1.09	0.00	74111	1.1	0.0		1.1	1.07	0.02		
1		Ni WER 1132R CDC T 6866	63795	257	0%	84.30%	0.41%	0.94	0.00	63795	0.9	0.0		0.9	0.92	0.02		
1		Ni WER 1132R CDC T 6868	67287	277	0%	84.79%	0.60%	0.99	0.00	67287	1.0	0.0		1.0	0.97	0.02		
1		Ni WER 1132R CDC T 6870	65625	411	1%	85.57%	0.65%	0.96	0.01	65625	0.9	0.0		1.0	0.95	0.02		
1		Ni WER 1132R CDC D 11201	64146	339	1%	86.04%	0.40%	0.94	0.01	64146	0.9	0.0		0.9	0.92	0.02		
1		Ni WER 1132R CDC D 11203	67530	286	0%	86.41%	0.74%	0.99	0.00	67530	1.0	0.0		1.0	0.97	0.02		
1		Ni WER 1132R CDC D 11205	65579	245	0%	83.53%	0.08%	0.96	0.00	65579	0.9	0.0		1.0	0.94	0.02		

2/9/17

ASC 2/10/17

Date of Run	Linear Dynamic Range (LDR)	$\Sigma(x_i)^2$	$\Sigma(x_i)$	D	n	Sample Reps	Detection Limit (ppb)
2/7/17	64	5.46E+03	128	1.31E+05	27	3.0	0.029
		x	y				Limit of Quantization
	Standard Uncertainty	5.0%	0.6%				0.014

1% HNO3	**Slope**	**Intercept**	
Slope	16332	1807	Intercept
Stdev	43	1068	Stdev
R^2	0.99995	2622	Stdev (Y)



Calibration Average	[Ni] (ppb)	Uncertainty	Average	Stdev	Rstdev	Blank Corrected Avg.
Standard 0	0	0.0	0	23		0
Standard 1	0.5	0.0	8933	152	1.70%	8933
Standard 2	1	0.1	17047	61	0.36%	17047
Standard 3	2	0.1	34293	37	0.11%	34293
Standard 4	4	0.2	69488	309	0.44%	69488
Standard 5	8	0.4	131205	389	0.30%	131205
Standard 6	16	0.8	263578	1732	0.66%	263578
Standard 7	32	1.6	529716	3542	0.67%	529716
Standard 8	64	3.2	1044355	4325	0.41%	1044355

Standard Addition Added (ppb):
9

Ran the standard addition solution previously and found it contained ~9 ppb instead of 10 ppb. Adjust the Standard Addition accordingly.

Average % Accuracy	3.2%
Average Standard % Recovery	99.5%
Average Sample % Recovery	100.6%

Average Precision 1.8%

					Instrument Data				**Calculated Raw Data**				Corrected For Dilution					
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty	% Error	% Recovery
1		Standard 0	0	23	0%	100.00%	0.25%	0.00	0.00	0	BMDL	-	0	BMDL	BMDL	-		
1		Standard 1	8933	152	2%	90.97%	0.67%	0.54	0.01	8933	0.4	0.1	0.5	0.5	0.44	0.10	12.7%	87.3%
1		Standard 2	17047	61	0%	96.05%	0.47%	1.03	0.00	17047	0.9	0.1	1	1.0	0.93	0.10	6.7%	93.3%
1		Standard 3	34293	37	0%	96.93%	0.36%	2.06	0.00	34293	2.0	0.1	2	2.1	1.99	0.10	0.5%	99.5%
1		Standard 4	69488	309	0%	94.02%	0.97%	4.18	0.02	69488	4.1	0.1	4	4.2	4.14	0.10	3.6%	103.6%
1		Standard 5	131205	389	0%	98.91%	0.69%	7.89	0.02	131205	7.9	0.1	8	7.9	7.92	0.10	1.0%	99.0%
1		Standard 6	263578	1732	1%	99.53%	0.30%	15.85	0.10	263578	16.0	0.1	16	15.9	16.03	0.15	0.2%	100.2%
1		Standard 7	529716	3542	1%	97.59%	1.09%	31.85	0.21	529716	32.3	0.2	32	31.9	32.32	0.25	1.0%	101.0%
1		Standard 8	1044355	4325	0%	99.57%	0.66%	62.80	0.26	1044355	63.8	0.3	64	62.8	63.83	0.31	0.3%	99.7%
1		Memory Blank	89	28	32%	93.83%	0.11%	0.01	0.00	89	BMDL	-	0	BMDL	BMDL	-		
1		QC Standard	165288	1471	1%	91.77%	0.79%	9.94	0.09	165288	10.0	0.1	10	9.9	10.01	0.13	0.1%	100.1%
1		OSU Effluent 01/05/17 D	3966	68	2%	92.94%	0.31%	0.24	0.00	3966	0.1	0.1	Method Blk (0)	0.2	0.13	0.10		
1		OSU Effluent 01/12/17 D	4450	82	2%	*77.70%*	0.21%	0.27	0.01	4450	0.2	0.1	VHW Ctl	0.3	0.16	0.10		
1		OSU Effluent 02/02/17 D	3634	19	1%	86.34%	0.86%	0.22	0.00	3634	0.1	0.1	0 (Con)	0.2	0.11	0.10		
1		Ni WER 1132R CDC T 6826	47579	835	2%	*70.64%*	1.19%	2.86	0.05	47579	2.8	0.1	2.1	2.9	2.80	0.11		
1		Ni WER 1132R CDC T 6829	94141	507	1%	*78.91%*	0.56%	5.66	0.03	94141	5.7	0.1	6	5.7	5.65	0.10		
1		Ni WER 1132R CDC T 6831	173715	917	1%	*79.13%*	0.38%	10.45	0.06	173715	10.5	0.1	12.2	10.5	10.53	0.11		
1		Ni WER 1132R CDC D 11161	47705	239	1%	82.10%	0.58%	2.87	0.01	47705	2.8	0.1	2.1	2.9	2.81	0.10		
1		Ni WER 1132R CDC D 11164	97906	376	0%	83.51%	0.84%	5.89	0.02	97906	5.9	0.1	6	5.9	5.88	0.10		
1		Ni WER 1132R CDC D 11166	180160	944	1%	83.46%	0.13%	10.83	0.06	180160	10.9	0.1	12.2	10.8	10.92	0.11		
1		Ni WER 1132R CDC D 11167	247806	1245	1%	84.50%	0.29%	14.90	0.08	247806	15.1	0.1	17.4	14.9	15.06	0.13		
1		Blank	2332	93	4%	99.25%	0.90%	0.14	0.01	2332	0.0	0.1	0	0.1	0.03	0.10		
1		QC Standard	183737	1516	1%	95.44%	0.47%	11.05	0.09	183737	11.1	0.1	10	11.1	11.14	0.13	11.4%	111.4%
1		Ni WER 1132R CDC T 6826	52157	592	1%	*79.22%*	0.64%	3.14	0.04	52157	3.1	0.1	2.1	3.1	3.08	0.10	4.8%	104.8%
1		Ni WER 1132R CDC D 11164 +S.	239566	831	0%	84.04%	0.56%	14.41	0.05	239566	14.6	0.1	12.9	14.4	14.56	0.11	-3.6%	96.4%
1		Ni WER 1132R CDC T 6866	72311	346	0%	84.30%	0.41%	4.35	0.02	72311	4.3	0.1	4.2	4.3	4.32	0.10		
1		Ni WER 1132R CDC T 6868	129664	247	0%	84.79%	0.60%	7.80	0.02	129664	7.8	0.1	8.5	7.8	7.83	0.10		
1		Ni WER 1132R CDC T 6870	248115	1422	1%	85.57%	0.65%	14.92	0.09	248115	15.1	0.1	17.4	14.9	15.08	0.13		
1		Ni WER 1132R CDC D 11201	69883	349	0%	86.04%	0.40%	4.20	0.02	69883	4.2	0.1	4.2	4.2	4.17	0.10		
1		Ni WER 1132R CDC D 11203	124902	449	0%	86.41%	0.74%	7.51	0.03	124902	7.5	0.1	8.5	7.5	7.54	0.10		
1		Ni WER 1132R CDC D 11205	236442	1488	1%	83.53%	0.08%	14.22	0.09	236442	14.4	0.1	17.4	14.2	14.37	0.13		

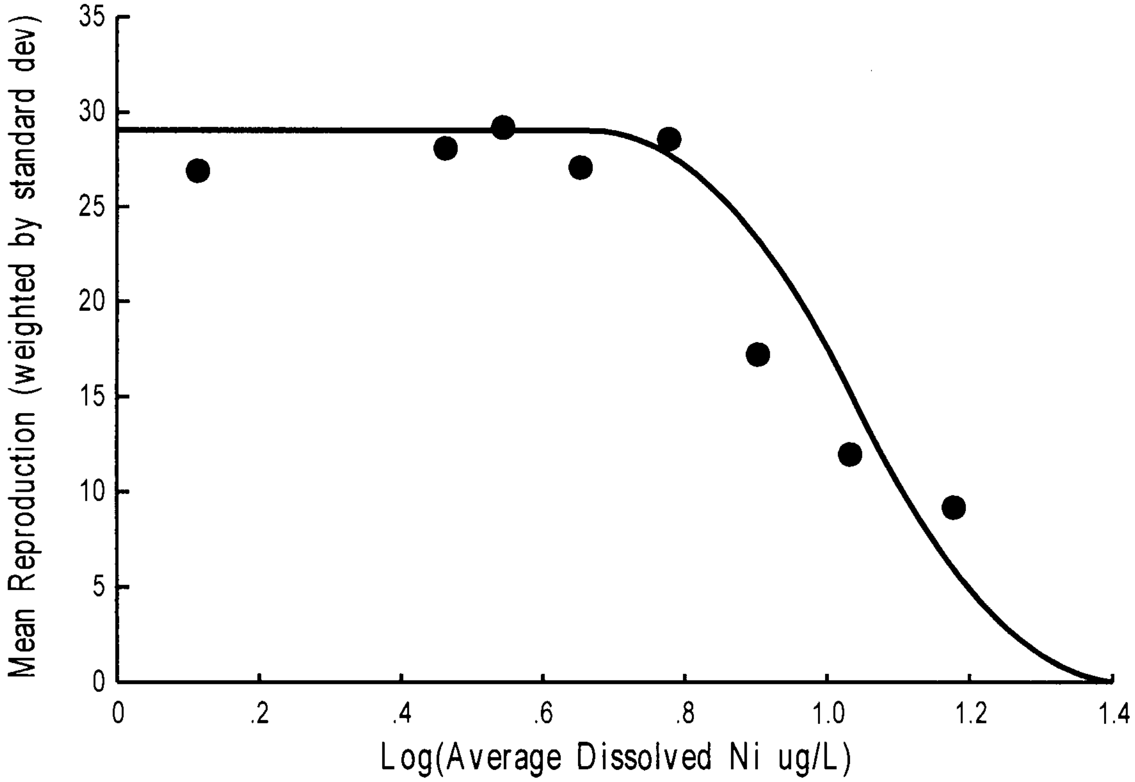
2/9/17

ASC 2/10/17

APPENDIX D

Raw Data

Chronic toxicity of a Ni-spiked simulated effluent - No DOC added: Ni WER 1132R



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.0515	1.0515	0.0337	0.9563	1.1296
S	2.689	2.689	0.621	1.060	4.252
Y0	27.98	27.98	1.47	25.26	32.84

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	11.040	9.043	13.479
20.0	8.028	6.060	10.635
10.0	6.837	4.759	9.823
5.0	6.103	3.953	9.423
0.0	4.640	2.414	8.919

ASC 3/27/17
ES 3/27/17

Chronic toxicity of a Ni-spiked simulated effluent - No DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	7	3698.228	528.318		
Regression	2	3697.048	1848.524	7829.	0.0000
Error	5	1.181	0.236		

Data Summary

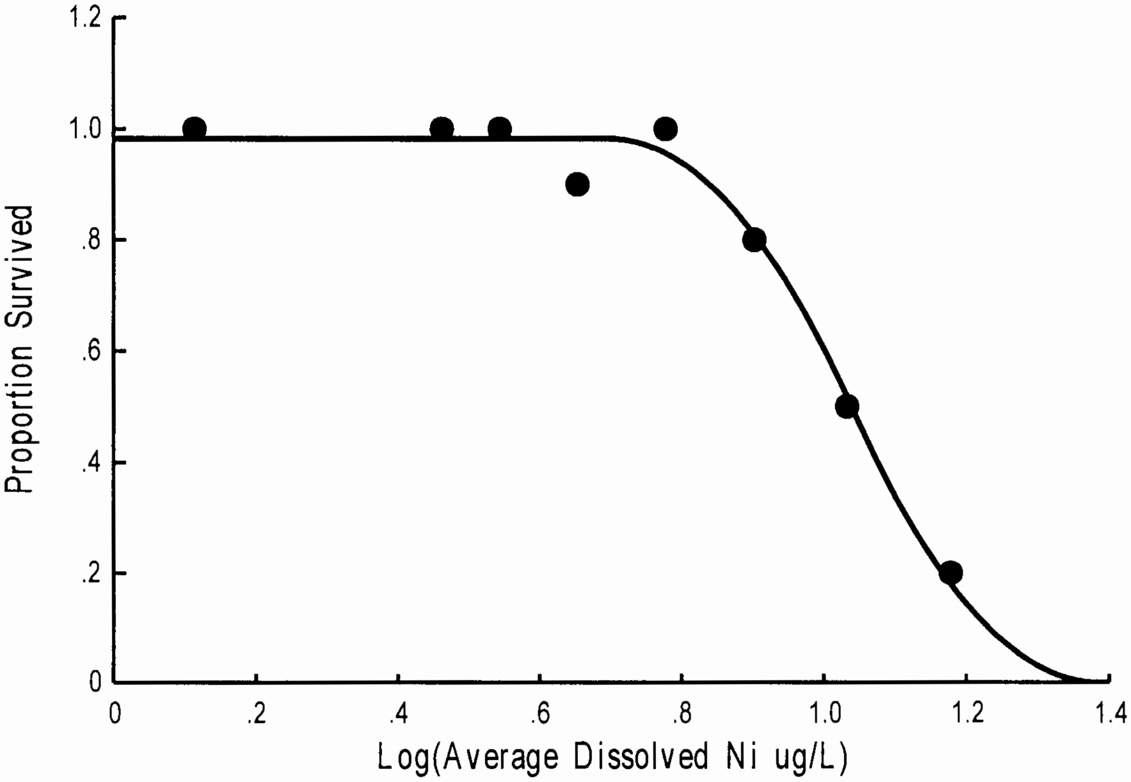
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	26.9000	29.0512	2.1512	6.7900
0.4624	28.1000	29.0512	0.9512	5.5470
0.5441	29.2000	29.0512	-0.1488	5.5340
0.6532	27.1000	29.0512	1.9512	9.0120
0.7782	28.6000	27.7737	-0.8263	2.2210
0.9031	17.2000	23.3148	6.1148	10.9000
1.0334	12.0000	15.2535	3.2535	6.4810
1.1790	9.2000	5.9265	-3.2735	5.9960

Error Summary

No Errors

ASC 3/27/17
ES 3/27/17

Chronic toxicity of a Ni-spiked simulated effluent - No DOC added: Ni WER 1132R



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.0525	1.0525	0.0135	1.0082	1.0774
S	3.211	3.211	0.379	1.936	3.884
Y0	0.9800	0.9800	0.0220	0.9257	1.0389

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	11.036	10.191	11.951
20.0	8.251	7.251	9.388
10.0	7.126	5.947	8.537
5.0	6.424	5.105	8.084
0.0	5.002	3.404	7.348

ASC 3/27/17
ES 3/27/17

Chronic toxicity of a Ni-spiked simulated effluent - No DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	7	0.62000	0.08857		
Regression	2	0.60954	0.30477	146.	0.0000
Error	5	0.01046	0.00209		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	1.0000	0.9823	-0.0177	1.
0.4624	1.0000	0.9823	-0.0177	1.
0.5441	1.0000	0.9823	-0.0177	1.
0.6532	0.9000	0.9823	0.0823	1.
0.7782	1.0000	0.9564	-0.0436	1.
0.9031	0.8000	0.8093	0.0093	1.
1.0334	0.5000	0.5176	0.0176	1.
1.1790	0.2000	0.1791	-0.0209	1.

Error Summary

No Errors

ASC 3/27/17
ES 3/27/17

CETIS Summary Report

Report Date: 11 Apr-17 14:05 (p 1 of 2)
Test Code: Ni WER 1132R CD | 05-7564-3253

Ceriodaphnia 7-d Survival and Reproduction Test — **OSU Aquatic Tox Lab**

Batch ID:	08-8046-5874	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	09 Jan-17 14:30	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	16 Jan-17 15:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d 1h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	25d 3h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) NO dissolved organic carbon (DOC) with B12 and Se. Concurrent control of very hard reconstituted water with B12 and Se.

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-8224-6664	7d Survival Rate	8	10.8	9.295	40.8%		Dunnett Multiple Comparison Test
08-9132-5268	Reproduction	8	>8	NA	27.5%		Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-9132-5268	Reproduction	PMSD	0.2747	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
1.2	Negative Contro	10	1	1	1	1	1	0	0	0.0%	0.0%
1.3	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
2.9		10	1	1	1	1	1	0	0	0.0%	0.0%
3.5		10	1	1	1	1	1	0	0	0.0%	0.0%
4.5		10	0.9	0.6738	1	0	1	0.1	0.3162	35.14%	10.0%
6		10	1	1	1	1	1	0	0	0.0%	0.0%
8		10	0.8	0.4984	1	0	1	0.1333	0.4216	52.7%	20.0%
10.8		10	0.5	0.123	0.877	0	1	0.1667	0.527	105.4%	50.0%
15.1		10	0.2	0	0.5016	0	1	0.1333	0.4216	210.8%	80.0%

Reproduction Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
1.2	Negative Contro	10	31.7	27.04	36.36	14	36	2.06	6.516	20.55%	0.0%
1.3	Dilution Water	10	26.9	22.04	31.76	19	39	2.147	6.79	25.24%	15.14%
2.9		10	28.1	24.13	32.07	18	34	1.754	5.547	19.74%	11.36%
3.5		10	29.2	25.24	33.16	19	36	1.75	5.534	18.95%	7.89%
4.5		10	27.1	20.65	33.55	3	35	2.85	9.012	33.25%	14.51%
6		10	28.6	27.01	30.19	25	32	0.7024	2.221	7.77%	9.78%
8		10	17.2	9.401	25	0	32	3.447	10.9	63.38%	45.74%
10.8		10	12	7.364	16.64	5	26	2.049	6.481	54.01%	62.15%
15.1		10	9.2	4.911	13.49	0	18	1.896	5.996	65.18%	70.98%

Dilution water = Simulated effluent (20% diluted) with no added DOC
Negative control = Very hard reconstituted water (concurrent control)

Analyst: ASC 4/11/17 QA: ES 4/11/17

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

7d Survival Rate Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.2	Negative Contro	1	1	1	1	1	1	1	1	1	1
1.3	Dilution Water	1	1	1	1	1	1	1	1	1	1
2.9		1	1	1	1	1	1	1	1	1	1
3.5		1	1	1	1	1	1	1	1	1	1
4.5		1	1	1	1	1	1	0	1	1	1
6		1	1	1	1	1	1	1	1	1	1
8		1	1	0	1	0	1	1	1	1	1
10.8		1	1	0	1	0	0	1	0	1	0
15.1		0	0	0	1	0	0	0	0	0	1

Reproduction Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.2	Negative Contro	36	33	36	30	34	33	36	14	32	33
1.3	Dilution Water	20	28	19	29	26	31	21	35	21	39
2.9		32	31	27	32	23	32	31	34	21	18
3.5		36	32	19	30	20	30	28	32	33	32
4.5		25	27	35	31	30	33	3	27	28	32
6		28	32	30	31	25	27	26	30	28	29
8		32	24	0	6	18	7	25	12	31	17
10.8		5	11	5	13	6	16	10	17	26	11
15.1		3	9	0	15	4	5	11	18	15	12

7d Survival Rate Binomials

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.2	Negative Contro	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1.3	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
2.9		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
3.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
4.5		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1
6		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
8		1/1	1/1	0/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
10.8		1/1	1/1	0/1	1/1	0/1	0/1	1/1	0/1	1/1	0/1
15.1		0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1

Analyst: ASC 4/11/17 QA: ES 4/11/17

CETIS Summary Report

Report Date: 11 Apr-17 14:04 (p 1 of 2)
Test Code: Ni WER 1132R CD | 05-7564-3253

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Batch ID:	08-8046-5874	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	09 Jan-17 14:30	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	16 Jan-17 15:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d 1h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	25d 3h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) NO dissolved organic carbon (DOC) with B12 and Se. Concurrent control of very hard reconstituted water with B12 and Se.

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-7309-0861	7d Survival Rate	1.2	>1.2	NA	NA		Fisher Exact Test
06-2275-7535	Reproduction	1.2	>1.2	NA	23.2%		Equal Variance t Two-Sample Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-2275-7535	Reproduction	PMSD	0.2324	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
1.2	Negative Contro	10	1	1	1	1	1	0	0	0.0%	0.0%
1.3	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
2.9		10	1	1	1	1	1	0	0	0.0%	0.0%
3.5		10	1	1	1	1	1	0	0	0.0%	0.0%
4.5		10	0.9	0.6738	1	0	1	0.1	0.3162	35.14%	10.0%
6		10	1	1	1	1	1	0	0	0.0%	0.0%
8		10	0.8	0.4984	1	0	1	0.1333	0.4216	52.7%	20.0%
10.8		10	0.5	0.123	0.877	0	1	0.1667	0.527	105.4%	50.0%
15.1		10	0.2	0	0.5016	0	1	0.1333	0.4216	210.8%	80.0%

Reproduction Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
1.2	Negative Contro	10	31.7	27.04	36.36	14	36	2.06	6.516	20.55%	0.0%
1.3	Dilution Water	10	26.9	22.04	31.76	19	39	2.147	6.79	25.24%	15.14%
2.9		10	28.1	24.13	32.07	18	34	1.754	5.547	19.74%	11.36%
3.5		10	29.2	25.24	33.16	19	36	1.75	5.534	18.95%	7.89%
4.5		10	27.1	20.65	33.55	3	35	2.85	9.012	33.25%	14.51%
6		10	28.6	27.01	30.19	25	32	0.7024	2.221	7.77%	9.78%
8		10	17.2	9.401	25	0	32	3.447	10.9	63.38%	45.74%
10.8		10	12	7.364	16.64	5	26	2.049	6.481	54.01%	62.15%
15.1		10	9.2	4.911	13.49	0	18	1.896	5.996	65.18%	70.98%

Analyst: ASC 4/11/17 QA: ES 4/11/17

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

7d Survival Rate Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.2	Negative Contro	1	1	1	1	1	1	1	1	1	1
1.3	Dilution Water	1	1	1	1	1	1	1	1	1	1
2.9		1	1	1	1	1	1	1	1	1	1
3.5		1	1	1	1	1	1	1	1	1	1
4.5		1	1	1	1	1	1	0	1	1	1
6		1	1	1	1	1	1	1	1	1	1
8		1	1	0	1	0	1	1	1	1	1
10.8		1	1	0	1	0	0	1	0	1	0
15.1		0	0	0	1	0	0	0	0	0	1

Reproduction Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.2	Negative Contro	36	33	36	30	34	33	36	14	32	33
1.3	Dilution Water	20	28	19	29	26	31	21	35	21	39
2.9		32	31	27	32	23	32	31	34	21	18
3.5		36	32	19	30	20	30	28	32	33	32
4.5		25	27	35	31	30	33	3	27	28	32
6		28	32	30	31	25	27	26	30	28	29
8		32	24	0	6	18	7	25	12	31	17
10.8		5	11	5	13	6	16	10	17	26	11
15.1		3	9	0	15	4	5	11	18	15	12

7d Survival Rate Binomials

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.2	Negative Contro	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1.3	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
2.9		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
3.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
4.5		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1
6		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
8		1/1	1/1	0/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
10.8		1/1	1/1	0/1	1/1	0/1	0/1	1/1	0/1	1/1	0/1
15.1		0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1

Analyst: ASC QA: ES
4/11/17 4/11/17

CETIS Analytical Report

Report Date:	28 Mar-17 12:23 (p 1 of 3)
Test Code:	Ni WER 1132R CD | 05-7564-3253

Ceriodaphnia 7-d Survival and Reproduction Test — **OSU Aquatic Tox Lab**

Analysis ID:	12-8224-6664	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Mar-17 12:22	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	08-8046-5874	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	09 Jan-17 14:30	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	16 Jan-17 15:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d 1h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	25d 3h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) NO dissolved organic carbon (DOC) with B12 and Se. Concurrent control of very hard reconstituted water with B12 and Se.

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	40.8%	8	10.8	9.295	

Dunnett Multiple Comparison Test

Control	vs	C-ug/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
1.3		2.9	0	2.386	0.169	18	0.8750	CDF	Non-Significant Effect
1.3		3.5	0	2.386	0.169	18	0.8750	CDF	Non-Significant Effect
1.3		4.5	0.7385	2.386	0.169	18	0.6009	CDF	Non-Significant Effect
1.3		6	0	2.386	0.169	18	0.8750	CDF	Non-Significant Effect
1.3		8	1.477	2.386	0.169	18	0.2682	CDF	Non-Significant Effect
1.3		10.8*	3.693	2.386	0.169	18	0.0014	CDF	Significant Effect
1.3		15.1*	5.908	2.386	0.169	18	<0.0001	CDF	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.699765	0.2428236	7	9.662	<0.0001	Significant Effect
Error	1.809427	0.02513094	72			
Total	3.509193		79			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	5.394	2.898	<0.0001	Unequal Variances
Variances	Levene Equality of Variance	16.04	2.898	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8439	0.9579	<0.0001	Non-normal Distribution

7d Survival Rate Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.3	Dilution Water	10	1	1	1	1	1	1	0	0.0%	0.0%
2.9		10	1	1	1	1	1	1	0	0.0%	0.0%
3.5		10	1	1	1	1	1	1	0	0.0%	0.0%
4.5		10	0.9	0.6738	1	1	0	1	0.1	35.14%	10.0%
6		10	1	1	1	1	1	1	0	0.0%	0.0%
8		10	0.8	0.4984	1	1	0	1	0.1333	52.7%	20.0%
10.8		10	0.5	0.123	0.877	0.5	0	1	0.1667	105.4%	50.0%
15.1		10	0.2	0	0.5016	0	0	1	0.1333	210.8%	80.0%

Analyst: ASC 4/11/17 QA: ES 4/11/17

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID: 12-8224-6664 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Mar-17 12:22 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Angular (Corrected) Transformed Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.3	Dilution Water	10	1.047	1.047	1.047	1.047	1.047	1.047	0	0.0%	0.0%
2.9		10	1.047	1.047	1.047	1.047	1.047	1.047	0	0.0%	0.0%
3.5		10	1.047	1.047	1.047	1.047	1.047	1.047	0	0.0%	0.0%
4.5		10	0.9948	0.8764	1.113	1.047	0.5236	1.047	0.05236	16.64%	5.0%
6		10	1.047	1.047	1.047	1.047	1.047	1.047	0	0.0%	0.0%
8		10	0.9425	0.7845	1.1	1.047	0.5236	1.047	0.06981	23.42%	10.0%
10.8		10	0.7854	0.588	0.9828	0.7854	0.5236	1.047	0.08727	35.14%	25.0%
15.1		10	0.6283	0.4704	0.7862	0.5236	0.5236	1.047	0.06981	35.14%	40.0%

7d Survival Rate Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.3	Dilution Water	1	1	1	1	1	1	1	1	1	1
2.9		1	1	1	1	1	1	1	1	1	1
3.5		1	1	1	1	1	1	1	1	1	1
4.5		1	1	1	1	1	1	0	1	1	1
6		1	1	1	1	1	1	1	1	1	1
8		1	1	0	1	0	1	1	1	1	1
10.8		1	1	0	1	0	0	1	0	1	0
15.1		0	0	0	1	0	0	0	0	0	1

Angular (Corrected) Transformed Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.3	Dilution Water	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047
2.9		1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047
3.5		1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047
4.5		1.047	1.047	1.047	1.047	1.047	1.047	0.5236	1.047	1.047	1.047
6		1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047	1.047
8		1.047	1.047	0.5236	1.047	0.5236	1.047	1.047	1.047	1.047	1.047
10.8		1.047	1.047	0.5236	1.047	0.5236	0.5236	1.047	0.5236	1.047	0.5236
15.1		0.5236	0.5236	0.5236	1.047	0.5236	0.5236	0.5236	0.5236	0.5236	1.047

7d Survival Rate Binomials

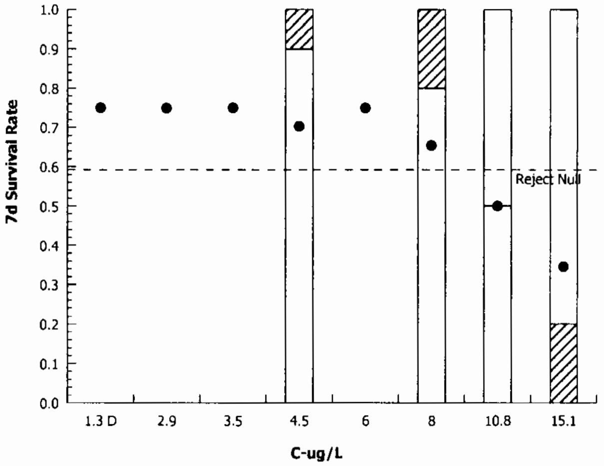
C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.3	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
2.9		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
3.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
4.5		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1
6		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
8		1/1	1/1	0/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
10.8		1/1	1/1	0/1	1/1	0/1	0/1	1/1	0/1	1/1	0/1
15.1		0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1

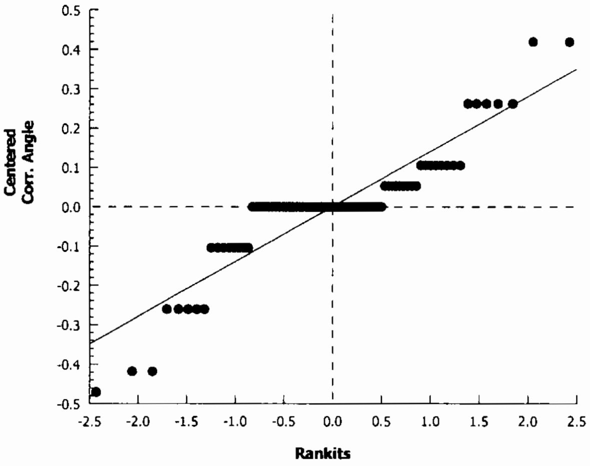
Analyst: ASC 4/11/17 QA: ES 4/11/17

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID: 12-8224-6664 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Mar-17 12:22 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Graphics





Analyst: ASC 4/11/17 QA: ES 4/11/17

CETIS Analytical Report

Report Date: 28 Mar-17 12:24 (p 1 of 2)
Test Code: Ni WER 1132R CD | 05-7564-3253

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID:	08-9132-5268	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Mar-17 12:24	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	08-8046-5874	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	09 Jan-17 14:30	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	16 Jan-17 15:00	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d 1h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	25d 3h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) NO dissolved organic carbon (DOC) with B12 and Se. Concurrent control of very hard reconstituted water with B12 and Se.

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	27.5%	8	>8	NA	

Steel Many-One Rank Sum Test

Control	vs	C-ug/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
1.3		2.9	114	75	2	18	0.9629	Asymp	Non-Significant Effect
1.3		3.5	117.5	75	3	18	0.9824	Asymp	Non-Significant Effect
1.3		4.5	113.5	75	3	18	0.9590	Asymp	Non-Significant Effect
1.3		6	115.5	75	4	18	0.9727	Asymp	Non-Significant Effect
1.3		8	78.5	75	1	18	0.0836	Asymp	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	26.9	15 - NL	Yes	Passes Acceptability Criteria
PMSD	0.2747	0.13 - 0.47	Yes	Passes Acceptability Criteria

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	3.491	3.2	0.0139	Outlier Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1006.683	201.3367	5	3.866	0.0046	Significant Effect
Error	2812.3	52.07963	54			
Total	3818.983		59			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	19.56	15.09	0.0015	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9479	0.9459	0.0125	Normal Distribution

Reproduction Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.3	Dilution Water	10	26.9	22.04	31.76	27	19	39	2.147	25.24%	0.0%
2.9		10	28.1	24.13	32.07	31	18	34	1.754	19.74%	-4.46%
3.5		10	29.2	25.24	33.16	31	19	36	1.75	18.95%	-8.55%
4.5		10	27.1	20.65	33.55	29	3	35	2.85	33.25%	-0.74%
6		10	28.6	27.01	30.19	28.5	25	32	0.7024	7.77%	-6.32%
8		10	17.2	9.401	25	17.5	0	32	3.447	63.38%	36.06%

Analyst: ASC 4/11/17 QA: ES 4/11/17

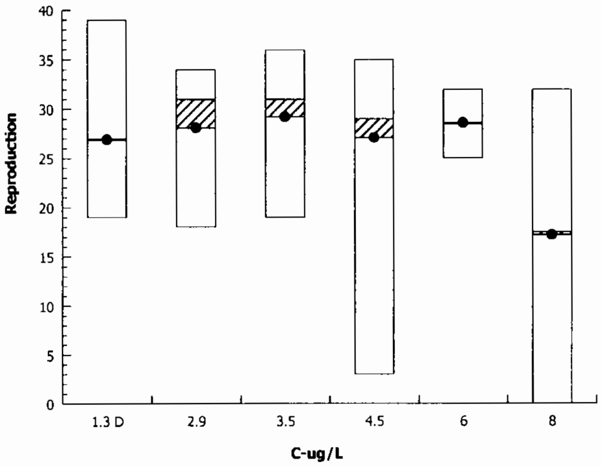
Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

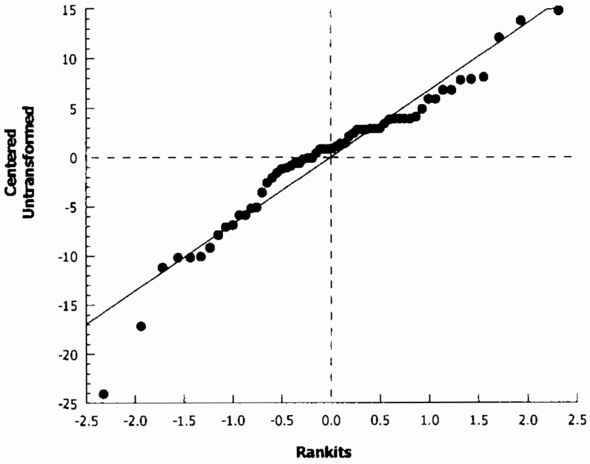
Analysis ID: 08-9132-5268 **Endpoint:** Reproduction **CETIS Version:** CETISv1.8.7
Analyzed: 28 Mar-17 12:24 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Reproduction Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.3	Dilution Water	20	28	19	29	26	31	21	35	21	39
2.9		32	31	27	32	23	32	31	34	21	18
3.5		36	32	19	30	20	30	28	32	33	32
4.5		25	27	35	31	30	33	3	27	28	32
6		28	32	30	31	25	27	26	30	28	29
8		32	24	0	6	18	7	25	12	31	17

Graphics





Analyst: ASC 4/11/17 QA: ES 4/11/17

Page:
OSU AquaTox QA Form No. 037
Revision: 0
Effective: 02/2010

DINAH

SUBJECT: TOXICITY DATA PACKAGE COVER SHEET

Test Type: Nickel WER - **Diluted Simulated Effluent (no DOC)**	Project Number: Ni WER 1132R CDC (NO DOC)
Test Substance: **NICKEL (as $NiCl_2 \times 6H_2O$)**	Species: *Ceriodaphnia dubia*
Dilution Water: Diluted Simulated Effluent RW (Reconstituted Lab H_2O) (w/B_{12} and Se) (at a 20% dilution from full Simulated)	Organism Lot or Batch Number: 010917 VHW 010917 SimEff
Concurrent Control Water: Very Hard RW (w/B_{12} and Se)	Age: < 24 hours Supplier: In-house
Date and Time Test Began: 1/9/17 @ 1430	Date and Time Test Ended: 1/16/17 @ 1500
Protocol Number: NIC-CD-CSR7d-005	Investigator(s): ES, TH, ASC

Background Information

Type of Test: Static-Renewal	pH Control?: Yes [✓ No] Type of Control: None	
Test Temperature: 25 ± 2 °C	Env. Chmbr/Bath #: 2 Test Chambers: 30- mL plastic	
Test Solution Vol.: 20 mL	Number of Replicates per Treatment: 10	
Length of Test: 7 days	Number of Organisms per Replicate: 1	
Type of Food and Quantity per Chamber: 0.3 mL Alg/YTC	Feeding Frequency: Once, before organism addition	
Test Substance Characterization Parameters and Frequency:	Hardness: Initiation, Day 3, 6, termination	Alkalinity: Initiation, Day 3, 6, termination
NH_3: Initiation pH: Daily	Conductivity: Daily TDS: Daily	TRC: Initiation
Test Conc.: 0 (Very Hard RW Conc.), 0 (Simulated RW no DOC/Control Dilution H_2O), 2.1, 2.9, 4.2, 6, 8.5, 12.2, 17.4 μg/L Nickel		

Reference Toxicant Data - Mean Reproduction

Test Dates: 1/9/17 to 1/16/17	LC_{50} or [✓ IC_{25}] (Circle): 817.8 mg/L Cl⁻
Hist. 95% Control Limits: 239.9 to 1181 mg/L Cl⁻	Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations

For seeding test, use neonates from simulated effluent monoboards for the control/dilution water and the nickel exposures. For the concurrent control, use neonates from Very Hard RW isolated adults from mass culture (due to availability).

Total volumes for each concentration will be prepared on different days. Control/dilution water will have B_{12} and Se nutrients.

Days 0, 3, 6: prepare 400 mL each day.

Days 1, 2, 4, 5: prepare 300 mL each day.

Prepare each concentration in a 500-mL graduated cylinder, although you will only be preparing **400 or 300 mL** on the specific days. Fill the cylinder with ~80% dilution water, then add appropriate amount of nickel stock to achieve desired concentration, then fill to line with dilution water. Mix well. Let solutions equilibrate for 3 hours at test temperature.

The Concurrent control with this test will be very-hard reconstituted water (VHW RW) with B_{12} and Se nutrients. Nutrients will be also added to the Diluted Simulated Effluent RW (no DOC) as the control/dilution water.

ATTENTION: Please be extra careful when pipetting and filling. Acid rinse and DI rinse the graduated cylinder after each day's use. Rinse out beakers with DI very well after each day.

METALS SAMPLING SCHEDULE: New Total and Dissolved (0.45 μm): Day 0, 3, 6; Old (Total and Dissolved composite): Day 4 and 7

READ PROTOCOL PRIOR TO WORKING ON THIS TEST. There will be measurements of TDS daily. Hardness/Alkalinity will be measured in multiple concentrations on multiple days (see protocol).

ASC 2/10/17 ES 1/17/17

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1132R CDC – no DOC

Chemical	Nickelous Chloride Hexahydrate $NiCl_2 \times 6H_2O$					
Chemical Manufacturer	J.T. Baker					
Chemical Lot #	L05582					
Nominal Stock Concentration	**20 mg/L Ni**					
Test Substance Stock Preparation Date and Time	Date: 12/15/16 @ 1100		Date: @		Date: @	
Date(s) Used	1/9/17					
	~~1/10/17~~ ①					
	③ 1/~~10~~/17					
	11 1/12/17					

PREPARATION OF TEST SOLUTIONS

Nominal Chemical Conc. (µg/L Ni)		**Nominal Stock Volume (µL)**	**Test Stock Volume (µL)**						
			Day 0	**Day ~~3~~** 2 ②	**Day ~~6~~** 3 ②				
0 (VHW RW) [1]		0	0	0	0				
#1	0 (Con-no DOC) [1]	0	0	0	0				
#2	2.1	42	42	42	42				
#3	2.9	58	58	58	58				
#4	4.2	84	84	84	84				
#5	6	120	120	120	120				
#6	8.5	170	170	170	170				
#7	12.2	244	244	244	244				
#8	17.4	348	348	348	348				
Total		**1066**	1066	1066	1066				
Total Volume per Treatment (400 mL)			400	400	400				
Dilution Water ID [1]			Simulated RW * 1485	Simulated RW * 1485	Simulated RW * 1485				
Concurrent Control Water ID [1]			VHW RW 1486	VHW RW 1486	VHW RW 1486				
Date			1/9/17	1/1~~0~~1 ③/17	1/12/17				
Time			1030	~~0950~~ ③ 1015	0945				
Initials			ES	TH	TH				

[1] Both the concurrent control (Very Hard RW) and the 20% Diluted Simulated Effluent RW (Control/dilution water) will have nutrients: B_{12} and Se

(Stock: Vitamin B_{12} 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B_{12})

(Stock: Na_2SeO_4 120 mg/L Stock = add 60 µL/L to achieve 3 µg/L Se)

Simulated RW *: water is full strength simulated effluent diluted by 20% with deionized water. No DOC added.

ES 1/17/17
ASC 2/10/17

① TH 1/10/17 wp ② ASC 1/11/17 CF ③ TH 1/11/17 E
(error was made in Ni addition. 100 mL of sim. added to each 300 mL)

Page:
OSU AquaTox QA Form No. 112
Revision: 0
Effective: 02/2010

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1132R CDC – no DOC

Chemical	**Nickelous Chloride Hexahydrate** **$NiCl_2 \times 6H_2O$**		
Chemical Manufacturer	JT Baker		
Chemical Lot #	L05582		
Nominal Stock Concentration	**20 mg/L Ni**		
Test Substance Stock Preparation Date and Time	Date: 12/15/16 @ 1100	Date: @	Date: @
Date(s) Used	1/10/17		

PREPARATION OF TEST SOLUTIONS

	Nominal Chemical Conc. (µg/L Ni)	**Nominal Stock Volume (µL)**	**Test Stock Volume (µL)**						
			Day 1	**Day ~~2~~** ①	**Day ~~4~~** ①	**Day ~~5~~** ①			
	0 (VHW RW) [1]	0	0						
#1	0 (Con-no DOC) [1]	0	0						
#2	2.1	31.5	31.5						
#3	2.9	43.5	43.5						
#4	4.2	63	63						
#5	6	90	90						
#6	8.5	127.5	127.5						
#7	12.2	183	183						
#8	17.4	261	261						
	Total	**799.5**	799.5						
	Total Volume per Treatment (300 mL)		300						
	Dilution Water ID [1]		Simulated RW * 1485	~~Simulated RW * ___~~ ①	~~Simulated RW * ___~~ ①	~~Simulated RW * ___~~ ①			
	Concurrent Control Water ID [1]		VHW RW 1486	~~VHW RW ___~~	~~VHW RW ___~~	~~VHW RW ___~~			
	Date		1/10/17						
	Time		0935						
	Initials		TH						

[1] Both the concurrent control (Very Hard RW) and the 20% Diluted Simulated Effluent RW (Control/dilution water) will have nutrients: B_{12} and Se

(Stock: Vitamin B_{12} 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B_{12})

(Stock: Na_2SeO_4 120 mg/L Stock = add 60 µL/L to achieve 3 µg/L Se)

Simulated RW *: water is full strength simulated effluent diluted by 20% with deionized water. No DOC added.

① ASC 1/12/17 CF

ES 1/17/17
ASC 2/10/17

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1132R CDC – no DOC

Chemical	**Nickelous Chloride Hexahydrate** **$NiCl_2 \times 6H_2O$**					
Chemical Manufacturer	JT Baker					
Chemical Lot #	LC5582					
Nominal Stock Concentration	**20 mg/L Ni**					
Test Substance Stock Preparation Date and Time	Date: 12/15/16 @ 1100		Date: @		Date: @	
Date(s) Used	1/13/17					
	1/14/17					
	1/15/17					

PREPARATION OF TEST SOLUTIONS

Nominal Chemical Conc. (µg/L Ni)		**Nominal Stock Volume (µL)**	**Test Stock Volume (µL)**						
			Day 4	**Day 5**	6				
0 (VHW RW) [1]		0	0	0	0				
#1	0 (Con-no DOC) [1]	0	0	0	0				
#2	2.1	26.3	26.3	26.3	26.3				
#3	2.9	36.3	36.3	36.3	36.3				
#4	4.2	52.5	52.5	52.5	52.5				
#5	6	75	75	75	75				
#6	8.5	106.3	106.3	106.3	106.3				
#7	12.2	152.5	152.5	152.5	152.5				
#8	17.4	217.5	217.5	217.5	217.5				
Total		**666.4**	666.4	666.4	666.4				
Total Volume per Treatment **(250 mL)**			250	250	250				
Dilution Water ID [1]			Simulated RW* 1485	Simulated RW* 1485	1485				
Concurrent Control Water ID [1]			VHW RW 1486	VHW RW 1486	1486				
Date			1/13/17	1/14/17	1/15/17				
Time			0955	0955	0955				
Initials			TH	TH	TH				

[1] Both the concurrent control (Very Hard RW) and the 20% Diluted Simulated Effluent RW (Control/dilution water) will have nutrients: B_{12} and Se

(Stock: Vitamin B_{12} 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B_{12})

(Stock: Na_2SeO_4 120 mg/L Stock = add 60 µL/L to achieve 3 µg/L Se)

Simulated RW *: water is full strength simulated effluent diluted by 20% with deionized water. No DOC added.

ES 1/17/17
ASC 2/10/17

Page:
OSU AquaTox QA Form No. 041
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1132R CDC – no DOC

Test Species: *C. dubia*

Conc. (µg/L Ni)		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter # All conc.	Remarks
Conc:	0 (VHW)										
pH		8.50	8.66	8.58	8.54	8.60	8.50	8.41		M16	
D.O. (mg/L)		8.7	8.6	8.7	8.3	8.6	8.9	8.8		M07	
Temp. (°C)		25	25	25	25	25	25	25		dig	
Cond. (µS/cm)		987	979	963	947	928	950	955		M03	
TDS (mg/L)		484	482	472	464	454	467	470		M03	
Hard. (mg/L)		328			288			284		titr	
Alk. (mg/L)		232			184			180		titr	
TRC (mg/L)		<0.05			NT			NT		PCII	
NH_3 (mg/L)		<1.0			NT			NT		M11	
#1	0 (Dil. Sim)										
pH		8.55	8.49	8.27	8.16	8.29	8.18	8.19			
D.O. (mg/L)		8.4	8.5	8.5	8.3	8.6	8.8	② 8.7 ~~9.2~~			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2350	2310	2300	2260	2230	2290	2320			
TDS (mg/L)		1189	1165	1161	1139	1129	1157	1168			
Hard. (mg/L)		328			272			248			
Alk. (mg/L)		432			380			328			
TRC (mg/L)		<0.05			NT			NT			
NH_3 (mg/L)		<1.0			NT			NT			
#2	2.1										
pH		8.57	8.51	8.28	8.18	8.28	8.20	8.20			
D.O. (mg/L)		8.4	8.4	8.4	8.3	8.6	8.7	8.7			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2360	2330	2310	2280	2270	2310	2320			
TDS (mg/L)		1194	1176	1167	1153	1151	1166	1172			
Hard. (mg/L)		~~328~~ ①									
Alk. (mg/L)		~~432~~ ①									
TRC (mg/L)											
NH_3 (mg/L)											
	Date:	1/9/17	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17			NT = not taken
	Time:	1340	1250	1320	1300	1255	1300	1305			
	Initials:	TH	TH	TH	MI	TH	TH	TH			

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, OSU TOX QA Form No. 011.

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

①TH 1/9/17 WP ②TH 1/15/17 E

ES 1/17/17

ASC 2/10/17

Page:
OSU TOX QA Form No. 041
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1132R CDC – no DOC

Test Species: *C. dubia*

Conc. (µg/L Ni)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter # All conc.	Remarks
#3 2.9										
pH	8.56	8.50	8.28	8.19	8.28	8.22	8.21			
D.O. (mg/L)	8.4	8.4	8.4	8.2	8.5	8.7	8.7			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2320	2310	2280	2250	2310	2330			
TDS (mg/L)	1190	1176	1168	1152	1138	1166	1177			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
#4 4.2										
pH	8.57	8.51	8.29	8.19	8.29	8.21	8.20			
D.O. (mg/L)	8.4	8.4	8.4	8.2	8.6	8.6	8.7			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2330	2310	2280	2250	2300	2320			
TDS (mg/L)	1190	1175	1167	1151	1139	1164	1175			
Hard. (mg/L)				272			270			
Alk. (mg/L)				372			356			
TRC (mg/L)				NT			NT			
NH_3 (mg/L)				NT			NT			
#5 6										
pH	8.56	8.50	8.28	8.16	8.30	8.20	8.20			
D.O. (mg/L)	8.4	8.4	8.4	8.2	8.5	8.6	8.7			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2320	2310	2280	2260	2300	2320			
TDS (mg/L)	1189	1173	1167	1150	1141	1164	1169			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	1/9/17	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17			NT = not taken
Time:	~~1240~~ 1340①	1250	1325	1300	1300	1300	1305			
Initials:	TH	TH	TH	MS	TH	TH	TH			

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, OSU TOX QA Form No. 011.
* Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting dilutions is assumed to also be 25°C.

①TH 1/9/17 E

ES 1/17/17
ASC 2/10/17

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1132R CDC – no DOC

Test Species: *C. dubia*

Conc. (**µg/L Ni**)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter # All conc.	Remarks
#6 8.5										
pH	8.57	8.50	8.28	8.17	8.30	8.22	8.19			
D.O. (mg/L)	8.4	8.4	8.4	8.2	8.5	8.6	8.7			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2330	2310	2290	2250	2300	2320			
TDS (mg/L)	1189	1174	1166	1150	1139	1164	1171			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
#7 12.2										
pH	8.55	8.49	8.28	8.18	8.32	8.22	8.20			
D.O. (mg/L)	8.4	8.4	8.4	8.2	8.5	8.6	8.6			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2320	2310	2290	2260	① 2300	2310			
TDS (mg/L)	1187	1172	1165	1146	1137	1165	1168			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
#8 17.4										
pH	8.57	8.51	8.29	8.18	8.33	8.21	8.21			
D.O. (mg/L)	8.4	8.4	8.4	8.2	8.6	8.6	8.6			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2320	2300	2290	2250	2300	2310			
TDS (mg/L)	1186	1172	1164	1151	1137	1164	1171			
Hard. (mg/L)				276			248			
Alk. (mg/L)				380			332			
TRC (mg/L)				NT			NT			
NH_3 (mg/L)				NT			NT			
Date:	1/9/17	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17			
Time:	1340	1250	1325	1300	1300	1300	1310			
Initials:	TH	TH	TH	M	TH	TH	TH			

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, OSU TOX QA Form No. 011.
* Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting dilutions is assumed to also be 25°C.

① TH 1/14/17 E

ES 1/17/17
ASC 2/10/17

Page:
OSU TOX QA Form No. 026
Revision: 0
Effective: 02/2010

SUBJECT: DAILY TOXICITY TEST LOG

Project Number: N. WER 1132R CDC

Test Species : C. dubia

General Comments – **Date:** 1/12/17 **Time:** 1200 **Tech:** TH

date	Intl	
1/12/17	TH	visable prec. on beakers sides and bottom
1/13/17	TH	visable particles in beakers #6 → #8 neonates smaller and
		pale.
1/14/17	TH	"
1/15/17	TH	Visable partiches in # 7 and # 8 and # 1

ASC 2/10/17 ES 1/17/17

OSU AquaTox QA Form No. 025
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1132R CDC – no DOC

Test Species: *C. dubia*

Conc. (µg/L Ni)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter # All Conc.	Remarks
Conc.: 0 VHW										
pH	8.75	8.68	8.65	8.68	8.61	8.58	8.51		M16	
D.O. (mg/L)	9.0	9.0	9.1	9.1	9.6	9.2	9.0		M07	
Cond. (µS/cm)	1028	1281	976	1038	1085	1074	1066		M03	
TDS (mg/L)	505	633	475	509	534	528	527		M03	
Temp. (°C)	25	25	25	25	25	25	25		dig	
#1 0 Dil. Sim										
pH	8.76	8.75	8.79	8.89	8.82	8.72	8.65			
D.O. (mg/L)	9.3	9.1	9.0	9.2	9.9	9.4	9.0			
Cond. (µS/cm)	2550	2390	~~2420~~ 2280 ①	2270	2360	2350	2410			
TDS (mg/L)	1293	1186	~~1210~~ ① 1154	1149	1191	1188	1220			
Temp. (°C)	25	25	25	25	25	25	25			
#2 2.1										
pH	8.74	8.74	8.77	8.88	8.82	8.77	8.69			
D.O. (mg/L)	9.1	9.0	8.80	9.2	9.7	9.2	9.0			
Cond. (µS/cm)	3040	2470	~~2290~~ 2420 ①	2290	2400	2490	~~2410~~ ②	2500		
TDS (mg/L)	1556	1252	① ~~1154~~ 1228	1158	1210	1260	~~1222~~ ②	1269		
Temp. (°C)	25	25	25	25	25	25	25			
#3 2.9										
pH	8.80	8.77	8.79	8.89	8.86	8.80	8.69			
D.O. (mg/L)	9.4	9.2	8.9	9.3	9.9	9.4	9.0			
Cond. (µS/cm)	2560	2440	2410	2280	2370	2370	2530			
TDS (mg/L)	1300	1235	1220	1152	1192	1200	1282			
Temp. (°C)	25	25	25	25	25	25	25			
#4 4.2										
pH	8.80	8.76	8.80	8.99	8.82	8.78	8.68			
D.O. (mg/L)	9.2	9.2	9.0	9.2	9.8	9.3	9.1			
Cond. (µS/cm)	2570	2370	2540	2300	2330	2550	2540			
TDS (mg/L)	1305	1201	1292	1165	~~1168~~ 1172 ②	1293	1288			
Temp. (°C)	25	25	25	25	25	25	25			
#5 6										
pH	8.77	8.73	8.82	8.90	8.85	8.81	8.70			
D.O. (mg/L)	9.4	9.1	9.0	9.4	9.9	9.4	9.2			
Cond. (µS/cm)	2640	2350	2420	2280	2350	2410	2510			
TDS (mg/L)	1339	1187	1225	1156	1189	1218	1274			
Temp. (°C)	25	25	25	25	25	25	25			
#6 8.5										
pH	8.76	8.71	8.80	8.93	8.85	8.80	8.73			
D.O. (mg/L)	9.2	9.0	9.0	9.5	9.9	9.3	9.2			
Cond. (µS/cm)	2580	2390	2350	2300	2360	2410	2440			
TDS (mg/L)	1310	1212	1190	1160	1195	1219	1236			
Temp. (°C)	25	25	25	25	25	25	25			
Date:	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17	1/16/17			
Time:	1340	1410	1345	1415	1340	1405	1530			
Initials:	TH	TH	TH	M)	TH	TH	TH			

① TH 1/12/17 WP ② TH 1/16/17 E

ASC 2/10/17 ES 1/17/17

OSU AquaTox QA Form No. 025
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1132R CDC – no DOC

Test Species: *C. dubia*

Conc. (µg/L Ni)		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter # All Conc.	Remarks
#7	12.2										
pH		8.75	8.68	8.75	8.89	8.81	8.79	8.69			
D.O. (mg/L)		9.3	9.2	9.1	9.5	9.9	9.3	9.2			
Cond. (µS/cm)		2430	2280	2420	2270	2320	2430	2590			
TDS (mg/L)		1231	1152	1224	1147	1171	1233	1316			
Temp. (°C)		25	25	25	25	25	25	25			
#8	17.4										
pH		8.74	8.74	8.81	8.90	8.80	8.76	8.69			
D.O. (mg/L)		9.2	9.1	9.1	9.4	9.8	9.3	9.2			
Cond. (µS/cm)		2830	2350	2290	2280	2350	2410	2360			
TDS (mg/L)		1444	1189	1157	1150	1186	1218	1195			
Temp. (°C)		25	25	25	25	25	25	25			
Date:		1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17	1/16/17			
Time:		1340	1410	1345	1415	1340	1405	1530			
Initials:		TH	TH	TH	MS	TH	TH	TH			

ASC 2/10/17 ES 1/17/17

SUBJECT: *CERIODAPHNIA DUBIA* CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1132R CDC – no DOC

		Number of Neonates Produced and Survival of Original Organisms												
Conc. µg/L Ni	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
0 (VHW)	1	0	0	0	0	0	0	0	0	0	0			
	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	1/3	0	0			
	4	1/6	1/8	1/7	1/4	1/6	1/6	1/6	1/1	1/6	1/5			
	5	0	2/8	0	1/1 2/10	2/11	0	2/11	2/10	0	2/13			
	6	2/12	0	2/~~4~~9②	0	0	2/11	0	0	2/8	0			
	7	3/18	3/17	3/20	3/15	3/17	3/16	3/19	0	3/18	3/15			
	8													
Total		36	33	36	30	34	33	36	14	32	33			
0 (Dil. Sim)	1	0	0	0	0	0	0	0	0	0	0			
#1	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	0	1/4	1/1	1/4	1/6	1/6	1/3	1/6	1/4	1/6			
	5	1/3	2/10	0	2/9	0	2/11	2/10	2/13	0	2/10			
	6	2/10	0	2/8	0	0	0	3/8	0	0	0			
	7	3/7	3/14	3/10	3/16	2/20	3/14	0	3/16	2/17	3/23			
	8													
Total		20	28	19	29	26	31	21	35	21	39			
2.1	1	0	0	0	0	0	0	0	0	0	0			
#2	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/7	1/4	1/6	1/4	1/6	1/7	1/5	1/5	1/6	1/6			
	5	0	2/10	0	2/11	0	2/13	①~~2~~ 2/11	1/2, 3/11	0	0			
	6	2/12	0	2/3	0	0	0	0	0	0	0			
	7	3/13	3/17	3/18	3/17	2/17	3/12	3/15	3/16	2/15	2/12			
	8													
Total		32	31	27	32	23	32	31	34	21	18			

DAY:	1	2	3	4	5	6	7	8		**Key to Symbols:**
Date:	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17	1/16/17			X = Original organism has died.
Time:	1335	1400	1315	1350	1245	1245	1410			M = Male.
Initials:	TH	TH	M	TH	TH	TH	TH			

①TH 1/14/17E ②TH 1/15/~~16~~17②E

ES 1/17/17
ASC 2/10/17

SUBJECT: *CERIODAPHNIA DUBIA* CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1132R CDC – no DOC

		Number of Neonates Produced and Survival of Original Organisms												
Conc. µg/L Ni	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
2.9	1	0	0	0	0	0	0	0	0	0	0			
#3	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/6	1/5	1/1	1/4	1/5	1/6	1/4	1/6	1/6	1/5			
	5	0	2/10	1/4	2/10	1/1	2/9	2/9	2/10	0	2/12			
	6	2/13	0	0	0	0	0	0	0	2/12	0			
	7	3/17	3/17	① ~~3~~ 2/14	3/16	2/14	3/15	3/15	3/16	3/15	3/15			
	8													
Total		36	32	19	30	20	30	28	32	33	32			
4.2	1	0	0	0	0	0	0	0	0	0	0			
#4	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/5	1/3	1/6	1/3	1/6	1/6	1/3 X	1/5	1/6	1/5			
	5	0	2/11	2/10	2/12	0	1/1 2/12		2/9	0	2/12			
	6	2/10	0	0	0	2/11	0		0	2/11	0			
	7	3/10	3/13	3/19	3/16	3/13	3/14		3/13	3/11	3/15			
	8													
Total		25	27	35	31	30	33	3	27	28	32			
6	1	0	0	0	0	0	0	0	0	0	0			
#5	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/7	1/5	1/5	1/5	1/4	1/3	1/5	1/5	1/6	1/5			
	5	0	1/1 2/10	1/1 2/9	2/10	2/8	1/3 2/8	2/8	2/9	0	2/11			
	6	2/11	0	0	0	0	0	0	0	2/11	0			
	7	3/10	3/16	3/15	3/16	3/13	3/13	3/13	3/16	3/11	3/13			
	8													
Total		28	32	30	31	25	27	26	30	28	29			

DAY:	1	2	3	4	5	6	7	8	
Date:	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17	1/16/17		
Time:	1335	1400	1330	1400	1250	1250	1420		
Initials:	TH	TH	TH	TH	TH	TH	TH		

Key to Symbols:

X = Original organism has died.

M = Male.

① TH 1/16/17 E

ES 1/17/17

ASC 2/10/17

Page:
OSU AquaTox QA Form No. 024
Revision: 0
Effective: 2/2010

SUBJECT: *CERIODAPHNIA DUBIA* CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1132R CDC – no DOC

		Number of Neonates Produced and Survival of Original Organisms												
Conc. µg/L Ni	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
8.5	1	0	0	0	0	0	0	0	0	0	0			
#6	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0 X	0	0	0	0	0	0	0			
	4	1/6	1/5		1/2	1/6	1/3	1/5	1/3	1/4	1/6			
	5	0	1/1, 2/7		0	0	0	2/9	2/9	0	2/5			
	6	2/14	0		2/4	2/12 X	2/4	0	0	2/13	0			
	7	3/12	3/11		0		0	3/11	0	3/14	3/6			
	8			↓		↓								
Total		32	24	0	6	18	7	25	12	31	17			
12.2	1	0	0	0	0	0	0	0	0	0	0			
#7	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/5	1/6	1/5	1/4	1/6	1/6	1/4	1/5	1/4	1/6			
	5	0	2/5	0 X	0	0	0	2/6	2/9	0	0			
	6	0	0		2/9	0 X	2/2 X	0	0	2/11	2/5 X			
	7	0	0		0			0	3/3 X	3/11				
	8			↓		↓	↓		↓		↓			
Total		5	11	5	13	6	16	10	17	26	11			
17.4	1	0	0	0	0	0	0	0	0	0	0			
#8	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/3	1/5	0 X	1/5	1/3	1/5	1/5	1/6	1/6	1/5			
	5	0	2/4		2/2	1/1 X	0 X	2/6	2/8	0	0			
	6	0 X	0 X		0			0 X	0	2/9 X	2/5			
	7				3/8				3/4 X		3/2			
	8	↓	↓	↓		↓	↓	↓	↓	↓				
Total		3	9	0	15	4	5	11	18	15	12			

DAY:	1	2	3	4	5	6	7	8		**Key to Symbols:**
Date:	1/10/17	1/11/17	1/12/17	1/13/17	1/14/17	1/15/17	1/16/17			X = Original organism has died.
Time:	1335	1400	1330	1415	1250	1250	1430			M = Male.
Initials:	TH	TH	TH	TH	TH	TH	TH			

ES 1/17/17
ASC 2/10/17

SUBJECT: DAILY TOXICITY TEST LOG

Project Number: Ni WER 1132R CDC – no DOC

Test Species: *C. dubia*

General Comments	For Concurrent VHW: Neonates obtained from (culture): CD010817 VHW on 1/9/17 TH For Diluted Simulated Effluent RW: Neonates obtained from (culture): CD123016 SIMEFF on 1/9/17 TH	Feeding
Test Day 0 1/9/17	3-hr Equilibrium Started at: 1030 ES 3-hr Equilibrium Ended at: 1335 TH Test Organisms Added at: 1430 TH Checked by: ES Total and Dissolved (0.45 µm) sampled at: 1440 TH	0.3 mL Alg/YTC @ 1405 TH
Test Day 1 1/10/17	3-hr Equilibrium Started at: 0935 TH 3-hr Equilibrium Ended at: 1250 TH	0.3 mL Alg/YTC @ 1315 TH
Test Day 2 1/11/17	3-hr Equilibrium Started at: 1015 TH 3-hr Equilibrium Ended at: 1320 TH	0.3 mL Alg/YTC @ 1330 TH
Test Day 3 1/12/17	3-hr Equilibrium Started at: 0945 TH 3-hr Equilibrium Ended at: 1250 TH New Total and Dissolved (0.45 µm) sampled at: 1330 MJ	0.3 mL Alg/YTC @ 1315 TH
Test Day 4 1/13/17	3-hr Equilibrium Started at: 0955 TH 3-hr Equilibrium Ended at: 1255 TH Old (comp) Total and Dissolved (0.45 µm) sampled at: 1445 TH	0.3 mL Alg/YTC @ 1330 TH
Test Day 5 1/14/17	3-hr Equilibrium Started at: 0955 TH 3-hr Equilibrium Ended at: 1255 TH	0.3 mL Alg/YTC @ 1315 TH
Test Day 6 1/15/17	3-hr Equilibrium Started at: 0955 TH 3-hr Equilibrium Ended at: 1300 TH New Total and Dissolved (0.45 µm) sampled at: 1330 TH	0.3 mL Alg/YTC @ 1320 TH
Test Day 7 1/16/17	Test Taken down at: ~~14~~ ① 1500 TH Old (comp) Total and Dissolved (0.45 µm) sampled at: 1545 TH	

① TH 1/16/17 E

ES 1/17/17
ASC 2/10/17

SIMULATED EFFLUENT FOR TESTING - *Ceriodaphnia dubia*

Total hardness = **400** mg/L as $CaCO_3$

Alkalinity = **400** mg/L as $CaCO_3$

Volume of water = **16** L (with 20% addition, will be total volume of 20-L)

Amount weighed		
4.5330	4.53299	grams $CaSO_4$ $2H_2O$
4.5280	4.52810	grams $MgSO_4$
3.1168	3.11680	grams KCl
8.7200	8.72000	grams NaCl
14.7840	14.78400	grams $NaHCO_3$

Estimated/Calculated Nominal (mg/L)	
52.2	Ca
56.5	Mg
467.3	Na
102.17	K
423.5	Cl
348.5	SO_4
488.1	HCO_3

	Manufacturer	Lot #
$CaSO_4 \cdot 2H_2O$	ACROS	A 0363568
$MgSO_4$	EMD	151020001
KCl	Alfa Aesar	E29Y012
NaCl	JT Baker	0000157485
$NaHCO_3$	Macron	0000134421

Recon. Water #: 1485

Test #: 1132R

Date/Time Prepared: 1/8/17 TH

Technician: TH

PREPARATION STEPS:

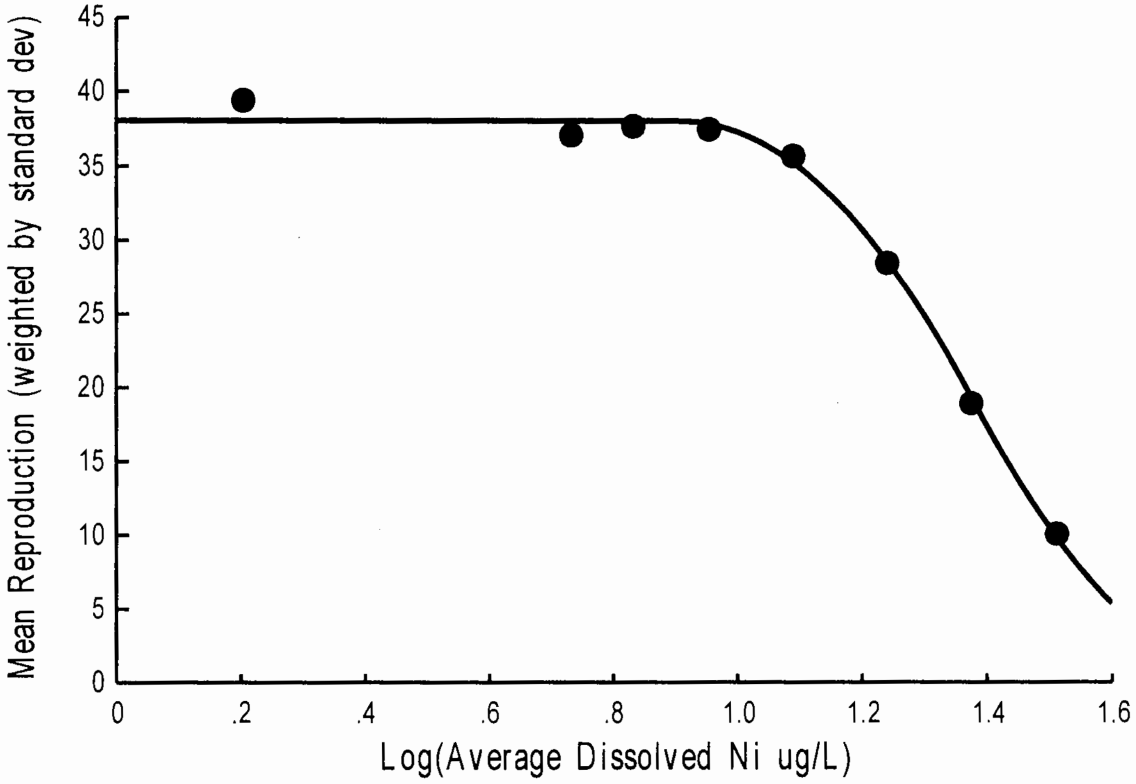
1/6/17 TH

1) In a gallon jar, add $CaSO_4 \cdot 2H_2O$ to 3-L DI. Put on stir plate. Mix overnight
2) In a gallon jar, add $MgSO_4$ to 3-L DI. Put on stir plate. Mix overnight
3) In 2-Liter Beaker, add KCl, NaCl, and $NaHCO_3$ to 2-L DI. Put on stir plate. Mix overnight
4) After the 3 containers have mixed overnight, combine and add 8 Liters DI for a total of 16 Liters in a 20-L cubi. 1/7/17 TH
5) Shake very well after combining. Put airstone (clean stone with clean tubing with a stopper to weigh it down) and bubble CO2 until pH is below 6.0 (preferably 5.6 - 5.8). 1/7/17 TH
6) Remove headspace in cubi and allow to sit overnight. 1/7/17 TH

1/8/17 TH

7) The next day, bubble air to bring pH up. After pH is above 8.3, add 4-L DI and mix well. This will be the "diluted simulated effluent".
8) Only remove enough volume for the day's use and remove headspace after each day.

Chronic toxicity of a nickel-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3847	1.3847	0.0111	1.3514	1.4082
S	2.249	2.249	0.158	1.718	2.528
Y0	37.93	37.93	0.43	36.95	39.18

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	23.98	22.46	25.60
20.0	16.095	14.640	17.694
10.0	13.166	11.580	14.969
5.0	11.422	9.755	13.374
0.0	8.106	6.337	10.369

ASC 3/24/17
ES 3/27/17

Chronic toxicity of a nickel-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	7	6872.9312	981.8473		
Regression	2	6872.8042	3436.4021	135252.	0.0000
Error	5	0.1270	0.0254		

Data Summary

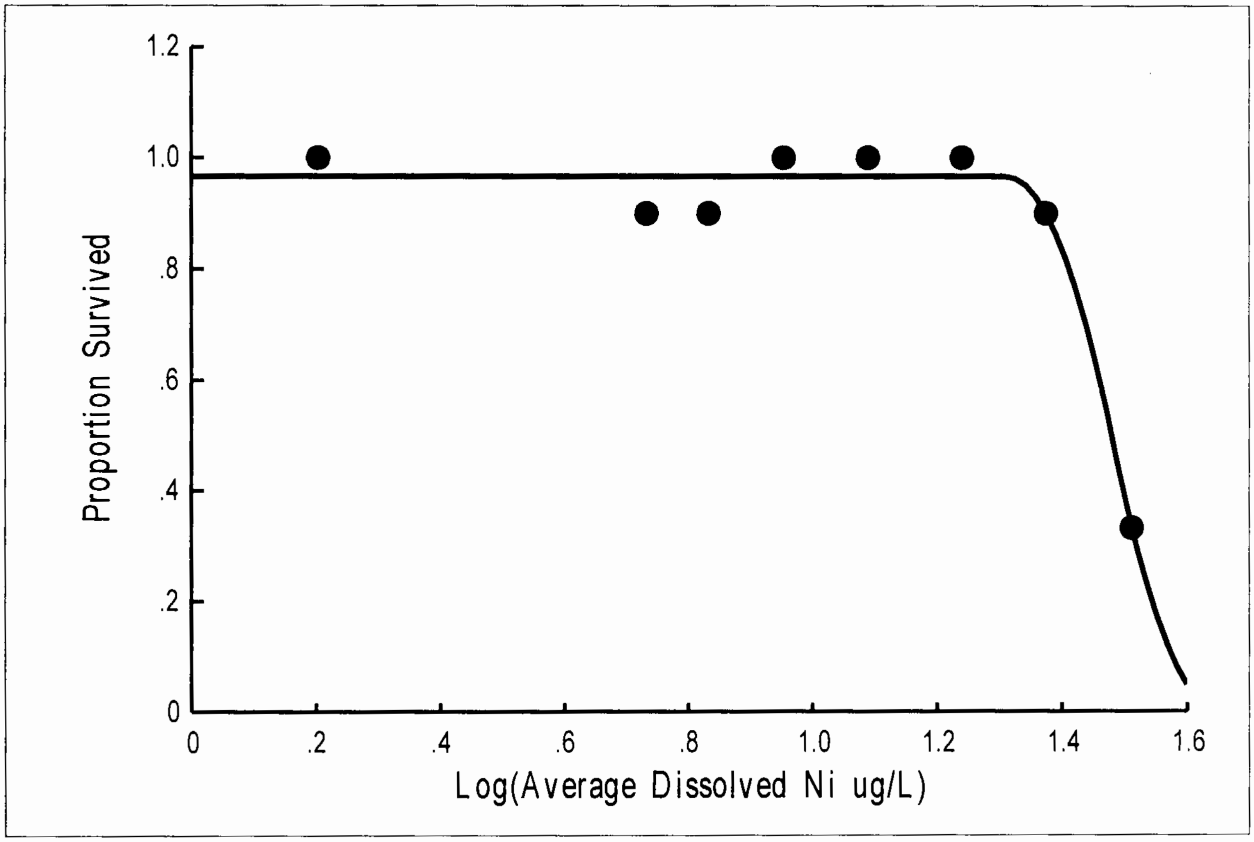
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	39.4000	38.0648	-1.3352	4.9260
0.7324	37.1000	38.0648	0.9648	13.1900
0.8325	37.7000	38.0648	0.3648	10.0700
0.9542	37.5000	37.8878	0.3878	2.3210
1.0899	35.7000	35.2513	-0.4487	3.7430
1.2405	28.5000	28.6229	0.1229	8.7340
1.3747	19.0000	19.4384	0.4384	7.6450
1.5119	10.1100	9.8541	-0.2559	7.6070

Error Summary

No Errors

ASC 3/24/17
ES 3/27/17

Chronic toxicity of a nickel-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4923	1.4923	0.0107	1.4553	1.5102
S	4.184	4.184	1.196	2.746	8.895
Y0	0.9667	0.9667	0.0211	0.9125	1.0209

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	30.39	28.53	32.37
20.0	26.28	23.56	29.31
10.0	24.42	21.19	28.15
5.0	23.19	19.62	27.41
0.0	20.46	15.92	26.30

03/27/2017 13:23

MED Toxicity Relationship Analysis Model, Version 1.30

ASC 3/27/17
ES 3/27/17

Chronic toxicity of a nickel-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	7	0.35768	0.05110		
Regression	2	0.34434	0.17217	64.6	0.0003
Error	5	0.01333	0.00267		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	1.0000	0.9667	-0.0333	1.
0.7324	0.9000	0.9667	0.0667	1.
0.8325	0.9000	0.9667	0.0667	1.
0.9542	1.0000	0.9667	-0.0333	1.
1.0899	1.0000	0.9667	-0.0333	1.
1.2405	1.0000	0.9667	-0.0333	1.
1.3747	0.9000	0.9000	0.0000	1.
1.5119	0.3333	0.3333	0.0000	1.

Error Summary

No Errors

ASC 3/27/17
ES 3/27/17

CETIS Summary Report

Report Date: 24 Mar-17 12:57 (p 1 of 2)
Test Code: Ni WER 1126 CDC | 06-2964-6522

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Batch ID:	17-2796-1237	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	16 Dec-16 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	23 Dec-16 14:30	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 23h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	28h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) with dissolved organic carbon (DOC)

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-6730-7280	7d Survival Rate	23.7	32.5	27.75	NA		Fisher Exact/Bonferroni-Holm Test
04-1468-3380	Reproduction	12.3	17.4	14.63	21.4%		Steel Many-One Rank Sum Test
17-7379-4994	Reproduction	12.3	17.4	14.63	17.9%		Wilcoxon/Bonferroni Adj Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-6730-7280	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
04-1468-3380	Reproduction	PMSD	0.2141	0.13 - 0.47	Yes	Passes Acceptability Criteria
17-7379-4994	Reproduction	PMSD	0.1793	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
1.6	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
5.4		10	0.9	0.6738	1	0	1	0.1	0.3162	35.14%	10.0%
6.8		10	0.9	0.6738	1	0	1	0.1	0.3162	35.14%	10.0%
9		10	1	1	1	1	1	0	0	0.0%	0.0%
12.3		10	1	1	1	1	1	0	0	0.0%	0.0%
17.4		10	1	1	1	1	1	0	0	0.0%	0.0%
23.7		10	0.9	0.6738	1	0	1	0.1	0.3162	35.14%	10.0%
32.5		9	0.3333	0	0.7177	0	1	0.1667	0.5	150.0%	66.67%

Reproduction Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
1.6	Dilution Water	10	39.4	35.88	42.92	31	47	1.558	4.926	12.5%	0.0%
5.4		10	37.1	27.66	46.54	0	44	4.173	13.19	35.57%	5.84%
6.8		10	37.7	30.5	44.9	10	45	3.183	10.07	26.7%	4.32%
9		10	37.5	35.84	39.16	33	41	0.7341	2.321	6.19%	4.82%
12.3		10	35.7	33.02	38.38	29	42	1.184	3.743	10.48%	9.39%
17.4		10	28.5	22.25	34.75	13	38	2.762	8.734	30.64%	27.66%
23.7		10	19	13.53	24.47	7	32	2.418	7.645	40.24%	51.78%
32.5		9	10.11	4.264	15.96	0	22	2.536	7.607	75.23%	74.34%

Analyst: ASC 3/24/17 QA: ES 3/27/17

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

7d Survival Rate Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	1	1	1	1	1	1	1	1	1	1
5.4		1	1	1	0	1	1	1	1	1	1
6.8		1	1	1	1	0	1	1	1	1	1
9		1	1	1	1	1	1	1	1	1	1
12.3		1	1	1	1	1	1	1	1	1	1
17.4		1	1	1	1	1	1	1	1	1	1
23.7		1	1	1	1	1	1	1	1	1	0
32.5		0	0	0	1	0	1	1	0	0	

Reproduction Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	33	44	37	39	40	47	31	40	39	44
5.4		41	44	38	0	38	42	42	40	43	43
6.8		43	41	45	43	10	42	38	38	37	40
9		39	33	41	35	37	39	39	36	38	38
12.3		32	38	37	37	42	35	33	29	39	35
17.4		25	13	36	16	37	36	29	27	38	28
23.7		11	20	17	32	19	21	29	21	7	13
32.5		5	4	8	15	0	22	20	5	12	

7d Survival Rate Binomials

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5.4		1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1
6.8		1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
9		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12.3		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
17.4		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
23.7		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
32.5		0/1	0/1	0/1	1/1	0/1	1/1	1/1	0/1	0/1	

Analyst: ASC 3/24/17 QA: ES 3/27/17

CETIS Analytical Report

Report Date: 24 Mar-17 12:57 (p 1 of 2)
Test Code: Ni WER 1126 CDC | 06-2964-6522

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID: 09-6730-7280
Analyzed: 24 Mar-17 12:57
Endpoint: 7d Survival Rate
Analysis: STP 2x2 Contingency Tables
CETIS Version: CETISv1.8.7
Official Results: Yes

Batch ID: 17-2796-1237
Start Date: 16 Dec-16 15:00
Ending Date: 23 Dec-16 14:30
Duration: 6d 23h
Test Type: Reproduction-Survival (7d)
Protocol: EPA/821/R-02-013 (2002)
Species: Ceriodaphnia dubia
Source: In-House Culture
Analyst: Allison Cardwell
Diluent: Simulated Effluent
Brine:
Age: <24h

Sample ID: 15-3887-2244
Sample Date: 15 Dec-16 11:00
Receive Date:
Sample Age: 28h
Code: 5BB953B4
Material: Nickel
Source: Chemical Reagent
Station:
Client: Internal Lab
Project:

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) with dissolved organic carbon (DOC)

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed		C > T	NA	NA	23.7	32.5	27.75	

Fisher Exact/Bonferroni-Holm Test

Sample	vs	Sample	Test Stat	P-Value	P-Type	Decision(α:5%)
1.6		5.4	0.5	1.0000	Exact	Non-Significant Effect
1.6		6.8	0.5	1.0000	Exact	Non-Significant Effect
1.6		9	1	1.0000	Exact	Non-Significant Effect
1.6		12.3	1	1.0000	Exact	Non-Significant Effect
1.6		17.4	1	1.0000	Exact	Non-Significant Effect
1.6		23.7	0.5	1.0000	Exact	Non-Significant Effect
1.6		32.5	0.003096	0.0217	Exact	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria

Data Summary

C-ug/L	Control Type	NR	R	NR + R	Prop NR	Prop R	%Effect
1.6	Dilution Water	10	0	10	1	0	0.0%
5.4		9	1	10	0.9	0.1	10.0%
6.8		9	1	10	0.9	0.1	10.0%
9		10	0	10	1	0	0.0%
12.3		10	0	10	1	0	0.0%
17.4		10	0	10	1	0	0.0%
23.7		9	1	10	0.9	0.1	10.0%
32.5		3	6	9	0.3333	0.6667	66.67%

7d Survival Rate Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	1	1	1	1	1	1	1	1	1	1
5.4		1	1	1	0	1	1	1	1	1	1
6.8		1	1	1	1	0	1	1	1	1	1
9		1	1	1	1	1	1	1	1	1	1
12.3		1	1	1	1	1	1	1	1	1	1
17.4		1	1	1	1	1	1	1	1	1	1
23.7		1	1	1	1	1	1	1	1	1	0
32.5		0	0	0	1	0	1	1	0	0	

Analyst: ASC 3/24/17 QA: ES 3/27/17

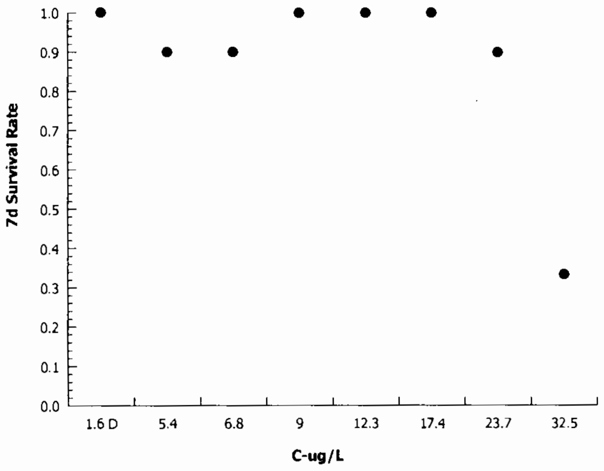
Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID: 09-6730-7280 **Endpoint:** 7d Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 24 Mar-17 12:57 **Analysis:** STP 2x2 Contingency Tables **Official Results:** Yes

7d Survival Rate Binomials

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5.4		1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1
6.8		1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
9		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12.3		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
17.4		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
23.7		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
32.5		0/1	0/1	0/1	1/1	0/1	1/1	1/1	0/1	0/1	

Graphics



Analyst: ASC QA: ES
3/24/17 3/27/17

CETIS Analytical Report

Report Date:	24 Mar-17 12:57 (p 1 of 4)
Test Code:	Ni WER 1126 CDC | 06-2964-6522

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID:	17-7379-4994	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Mar-17 12:57	**Analysis:**	Nonparametric-Multiple Comparison	**Official Results:**	Yes

Batch ID:	17-2796-1237	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	16 Dec-16 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	23 Dec-16 14:30	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 23h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	28h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) with dissolved organic carbon (DOC)

Sample Note: Chemical: Nickelous Chloride Hexhydrate ($NiCl2 \times 6H2O$). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	17.9%	12.3	17.4	14.63	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-ug/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
1.6		5.4	100	NA	2	17	1.0000	Exact	Non-Significant Effect
1.6		6.8	106.5	NA	2	18	1.0000	Exact	Non-Significant Effect
1.6		9	88	NA	3	18	0.6181	Exact	Non-Significant Effect
1.6		12.3	80.5	NA	3	18	0.1934	Exact	Non-Significant Effect
1.6		17.4*	64.5	NA	1	18	0.0034	Exact	Significant Effect
1.6		23.7*	56	NA	0	18	<0.0001	Exact	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	39.4	15 - NL	Yes	Passes Acceptability Criteria
PMSD	0.1793	0.13 - 0.47	Yes	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3601.714	600.2856	6	14.57	<0.0001	Significant Effect
Error	2555.156	41.21218	62			
Total	6156.87		68			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	32	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8897	0.952	<0.0001	Non-normal Distribution

Reproduction Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.6	Dilution Water	10	39.4	35.88	42.92	39.5	31	47	1.558	12.5%	0.0%
5.4		9	41.22	39.56	42.89	42	38	44	0.7222	5.26%	-4.63%
6.8		10	37.7	30.5	44.9	40.5	10	45	3.183	26.7%	4.32%
9		10	37.5	35.84	39.16	38	33	41	0.7341	6.19%	4.82%
12.3		10	35.7	33.02	38.38	36	29	42	1.184	10.48%	9.39%
17.4		10	28.5	22.25	34.75	28.5	13	38	2.762	30.64%	27.66%
23.7		10	19	13.53	24.47	19.5	7	32	2.418	40.24%	51.78%

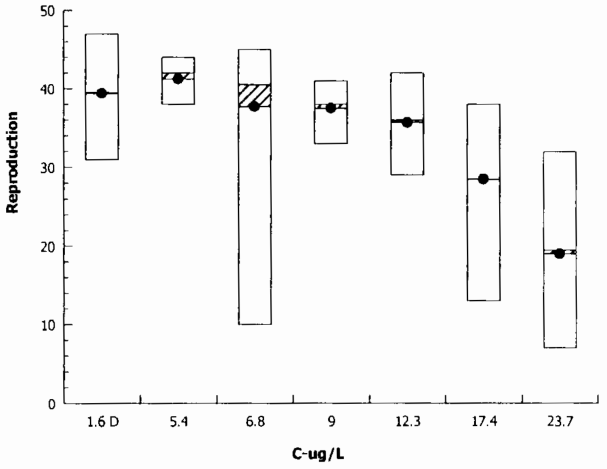
Analyst: ASC 3/24/17 QA: ES 3/27/17

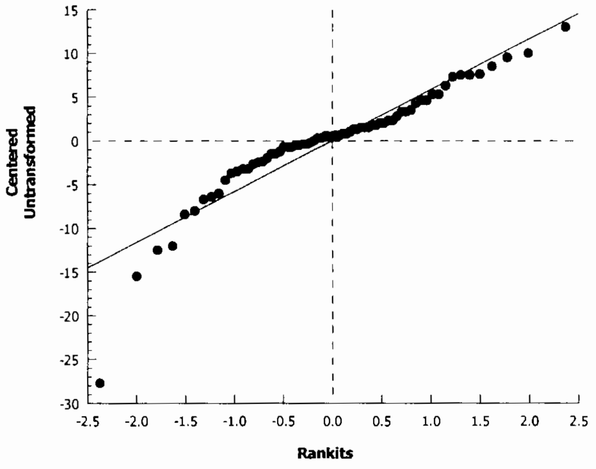
Analysis ID: 17-7379-4994 **Endpoint:** Reproduction **CETIS Version:** CETISv1.8.7
Analyzed: 24 Mar-17 12:57 **Analysis:** Nonparametric-Multiple Comparison **Official Results:** Yes

Reproduction Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	33	44	37	39	40	47	31	40	39	44
5.4		41	44	38	Outlier	38	42	42	40	43	43
6.8		43	41	45	43	10	42	38	38	37	40
9		39	33	41	35	37	39	39	36	38	38
12.3		32	38	37	37	42	35	33	29	39	35
17.4		25	13	36	16	37	36	29	27	38	28
23.7		11	20	17	32	19	21	29	21	7	13

Graphics





Analyst: ASC 3/24/17 QA: ES 3/27/17

CETIS Analytical Report

Report Date: 24 Mar-17 12:57 (p 3 of 4)
Test Code: Ni WER 1126 CDC | 06-2964-6522

Ceriodaphnia 7-d Survival and Reproduction Test **OSU Aquatic Tox Lab**

Analysis ID:	04-1468-3380	**Endpoint:**	Reproduction	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Mar-17 12:57	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Batch ID:	17-2796-1237	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Allison Cardwell
Start Date:	16 Dec-16 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Simulated Effluent
Ending Date:	23 Dec-16 14:30	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	6d 23h	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	15-3887-2244	**Code:**	5BB953B4	**Client:**	Internal Lab
Sample Date:	15 Dec-16 11:00	**Material:**	Nickel	**Project:**	
Receive Date:		**Source:**	Chemical Reagent		
Sample Age:	28h	**Station:**			

Batch Note: Control/Dilution water: Simulated Effluent (20% diluted) with dissolved organic carbon (DOC)

Sample Note: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	21.4%	12.3	17.4	14.63	

Steel Many-One Rank Sum Test

Control	vs	C-ug/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
1.6		5.4	110	74	2	18	0.9366	Asymp	Non-Significant Effect
1.6		6.8	106.5	74	2	18	0.8859	Asymp	Non-Significant Effect
1.6		9	88	74	3	18	0.3191	Asymp	Non-Significant Effect
1.6		12.3	80.5	74	3	18	0.1282	Asymp	Non-Significant Effect
1.6		17.4*	64.5	74	1	18	0.0059	Asymp	Significant Effect
1.6		23.7*	56	74	0	18	0.0006	Asymp	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	39.4	15 - NL	Yes	Passes Acceptability Criteria
PMSD	0.2141	0.13 - 0.47	Yes	Passes Acceptability Criteria

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	4.822	3.258	<0.0001	Outlier Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3214.771	535.7952	6	8.264	<0.0001	Significant Effect
Error	4084.5	64.83334	63			
Total	7299.271		69			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	30.3	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.7998	0.9526	<0.0001	Non-normal Distribution

Reproduction Summary

C-ug/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.6	Dilution Water	10	39.4	35.88	42.92	39.5	31	47	1.558	12.5%	0.0%
5.4		10	37.1	27.66	46.54	41.5	0	44	4.173	35.57%	5.84%
6.8		10	37.7	30.5	44.9	40.5	10	45	3.183	26.7%	4.32%
9		10	37.5	35.84	39.16	38	33	41	0.7341	6.19%	4.82%
12.3		10	35.7	33.02	38.38	36	29	42	1.184	10.48%	9.39%
17.4		10	28.5	22.25	34.75	28.5	13	38	2.762	30.64%	27.66%
23.7		10	19	13.53	24.47	19.5	7	32	2.418	40.24%	51.78%

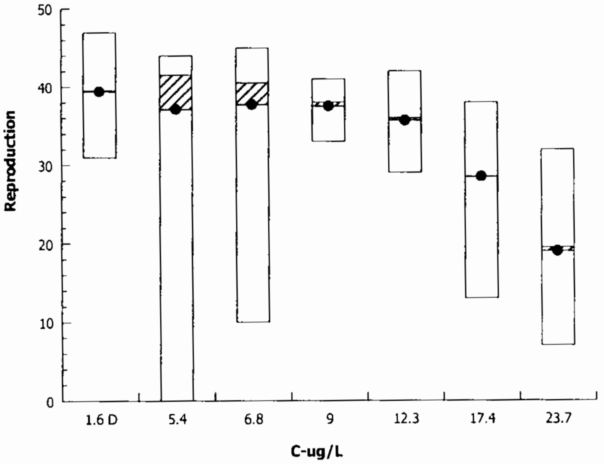
Analyst: ASC 3/24/17 QA: ES 3/27/17

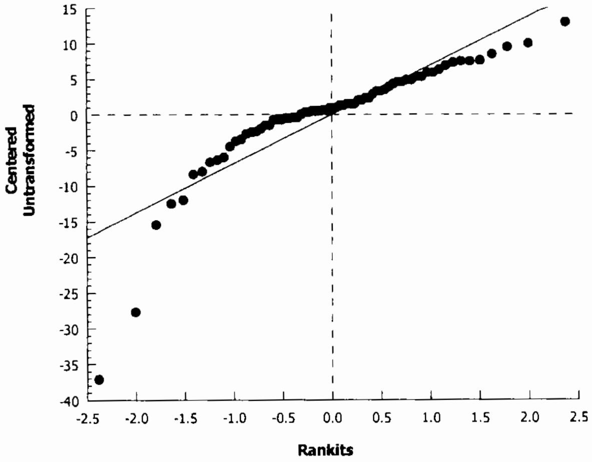
Analysis ID: 04-1468-3380 **Endpoint:** Reproduction **CETIS Version:** CETISv1.8.7
Analyzed: 24 Mar-17 12:57 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Reproduction Detail

C-ug/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
1.6	Dilution Water	33	44	37	39	40	47	31	40	39	44
5.4		41	44	38	0	38	42	42	40	43	43
6.8		43	41	45	43	10	42	38	38	37	40
9		39	33	41	35	37	39	39	36	38	38
12.3		32	38	37	37	42	35	33	29	39	35
17.4		25	13	36	16	37	36	29	27	38	28
23.7		11	20	17	32	19	21	29	21	7	13

Graphics





Analyst: ASC 3/24/17 QA: ES 3/27/17

CETIS Test Data Worksheet

Report Date: 24 Mar-17 12:20 (p 1 of 3)
Test Code: 06-2964-6522/Ni WER 1126 CDC

Ceriodaphnia 7-d Survival and Reproduction Test — **OSU Aquatic Tox Lab**

Start Date: 16 Dec-16 15:00 **Species:** Ceriodaphnia dubia **Sample Code:** 5BB953B4
End Date: 23 Dec-16 14:30 **Protocol:** EPA/821/R-02-013 (2002) **Sample Source:** Chemical Reagent
Sample Date: 15 Dec-16 11:00 **Material:** Nickel **Sample Station:**

Batch Notes: Control/Dilution water: Simulated Effluent (20% diluted) with dissolved organic carbon (DOC)

Sample Notes: Chemical: Nickelous Chloride Hexhydrate (NiCl2 x 6H2O). Manufacturer: JT Baker. Lot: L05582. Nominal Stock concentration: 20 mg/L Ni.

C-ug/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Neonates	Male	8dSurvival	Notes
1.6	D	1	43	1	1	1	1	1	1	1	1	33	0		
1.6	D	2	76	1	1	1	1	1	1	1	1	44	0		
1.6	D	3	22	1	1	1	1	1	1	1	1	37	0		
1.6	D	4	12	1	1	1	1	1	1	1	1	39	0		
1.6	D	5	66	1	1	1	1	1	1	1	1	40	0		
1.6	D	6	10	1	1	1	1	1	1	1	1	47	0		
1.6	D	7	67	1	1	1	1	1	1	1	1	31	0		
1.6	D	8	56	1	1	1	1	1	1	1	1	40	0		
1.6	D	9	64	1	1	1	1	1	1	1	1	39	0		
1.6	D	10	35	1	1	1	1	1	1	1	1	44	0		
5.4		1	33	1	1	1	1	1	1	1	1	41	0		
5.4		2	34	1	1	1	1	1	1	1	1	44	0		
5.4		3	1	1	1	1	1	1	1	1	1	38	0		
5.4		4	39	1	1	0	0	0	0	0	0	0	0		
5.4		5	27	1	1	1	1	1	1	1	1	38	0		
5.4		6	71	1	1	1	1	1	1	1	1	42	0		
5.4		7	47	1	1	1	1	1	1	1	1	42	0		
5.4		8	50	1	1	1	1	1	1	1	1	40	0		
5.4		9	23	1	1	1	1	1	1	1	1	43	0		
5.4		10	30	1	1	1	1	1	1	1	1	43	0		
6.8		1	7	1	1	1	1	1	1	1	1	43	0		
6.8		2	73	1	1	1	1	1	1	1	1	41	0		
6.8		3	11	1	1	1	1	1	1	1	1	45	0		
6.8		4	17	1	1	1	1	1	1	1	1	43	0		
6.8		5	45	1	1	1	1	1	1	0	0	10	0		
6.8		6	20	1	1	1	1	1	1	1	1	42	0		
6.8		7	75	1	1	1	1	1	1	1	1	38	0		
6.8		8	24	1	1	1	1	1	1	1	1	38	0		
6.8		9	36	1	1	1	1	1	1	1	1	37	0		
6.8		10	6	1	1	1	1	1	1	1	1	40	0		

Analyst: ASC 3/24/17 QA: ES 3/27/17

CETIS Test Data Worksheet

Test Code: 06-2964-6522/Ni WER 1126 CDC

C-ug/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Neonates	Male	8dSurvival	Notes
9		1	46	1	1	1	1	1	1	1	1	39	0		
9		2	61	1	1	1	1	1	1	1	1	33	0		
9		3	55	1	1	1	1	1	1	1	1	41	0		
9		4	2	1	1	1	1	1	1	1	1	35	0		
9		5	29	1	1	1	1	1	1	1	1	37	0		
9		6	74	1	1	1	1	1	1	1	1	39	0		
9		7	9	1	1	1	1	1	1	1	1	39	0		
9		8	70	1	1	1	1	1	1	1	1	36	0		
9		9	49	1	1	1	1	1	1	1	1	38	0		
9		10	26	1	1	1	1	1	1	1	1	38	0		
12.3		1	37	1	1	1	1	1	1	1	1	32	0		
12.3		2	3	1	1	1	1	1	1	1	1	38	0		
12.3		3	21	1	1	1	1	1	1	1	1	37	0		
12.3		4	63	1	1	1	1	1	1	1	1	37	0		
12.3		5	19	1	1	1	1	1	1	1	1	42	0		
12.3		6	78	1	1	1	1	1	1	1	1	35	0		
12.3		7	13	1	1	1	1	1	1	1	1	33	0		
12.3		8	5	1	1	1	1	1	1	1	1	29	0		
12.3		9	28	1	1	1	1	1	1	1	1	39	0		
12.3		10	31	1	1	1	1	1	1	1	1	35	0		
17.4		1	77	1	1	1	1	1	1	1	1	25	0		
17.4		2	44	1	1	1	1	1	1	1	1	13	0		
17.4		3	53	1	1	1	1	1	1	1	1	36	0		
17.4		4	65	1	1	1	1	1	1	1	1	16	0		
17.4		5	4	1	1	1	1	1	1	1	1	37	0		
17.4		6	79	1	1	1	1	1	1	1	1	36	0		
17.4		7	51	1	1	1	1	1	1	1	1	29	0		
17.4		8	62	1	1	1	1	1	1	1	1	27	0		
17.4		9	38	1	1	1	1	1	1	1	1	38	0		
17.4		10	68	1	1	1	1	1	1	1	1	28	0		
23.7		1	41	1	1	1	1	1	1	1	1	11	0		
23.7		2	60	1	1	1	1	1	1	1	1	20	0		
23.7		3	72	1	1	1	1	1	1	1	1	17	0		
23.7		4	25	1	1	1	1	1	1	1	1	32	0		
23.7		5	58	1	1	1	1	1	1	1	1	19	0		
23.7		6	32	1	1	1	1	1	1	1	1	21	0		
23.7		7	16	1	1	1	1	1	1	1	1	29	0		

Analyst: ASC 3/24/17

QA: ES 3/27/17

CETIS Test Data Worksheet

C-ug/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Neonates	Male	8dSurvival	Notes
23.7		8	48	1	1	1	1	1	1	1	1	21	0		
23.7		9	15	1	1	1	1	1	1	1	1	7	0		
23.7		10	59	1	1	1	1	1	1	1	0	13	0		
32.5		1	69	1	1	1	1	1	1	0	0	5	0		
32.5		2	42	1	1	1	1	0	0	0	0	4	0		
32.5		3	14	1	1	1	1	1	1	0	0	8	0		
32.5		4	54	1	1	1	1	1	1	1	1	15	0		
32.5		5	40	1	1	0	0	0	0	0	0	0	0		
32.5		6	8	1	1	1	1	1	1	1	1	22	0		
32.5		7	52	1	1	1	1	1	1	1	1	20	0		
32.5		8	57	1	1	1	1	1	1	0	0	5	0		
32.5		9	18	1	1	1	1	1	1	0	0	12	0		

Analyst: ASC 3/24/17

QA: ES 3/27/17

Page:
OSU AquaTox QA Form No. 037
Revision: 0
Effective: 02/2010

DINAH

SUBJECT: TOXICITY DATA PACKAGE COVER SHEET

Test Type: Nickel WER - **Diluted Sim. Effluent (WITH DOC)**	Project Number: Ni WER 1126 CDC (WITH DOC)
Test Substance: **NICKEL (as $NiCl_2 \times 6H_2O$)**	Species: *Ceriodaphnia dubia*
Dilution Water: With DOC-Diluted Simulated Effluent RW (Reconstituted Lab H_2O) (w/B_{12} and Se)	Organism Lot or Batch Number: CD 121616 Simulated Effluent
Concurrent Control Water: None	Age: < 24 hours Supplier: In-house
Date and Time Test Began: 12/16/16 @ 1500	Date and Time Test Ended: 12/23/16 @ 1430
Protocol Number: NIC-CD-CSR7d-005	Investigator(s): ES, TH, MS, ASC

Background Information

Type of Test: Static-Renewal	pH Control?: Yes [✓ No] Type of Control: None	
Test Temperature: 25 ± 2 °C	Env. Chmbr/Bath #: 2 Test Chambers: 30- mL plastic	
Test Solution Vol.: 20 mL	Number of Replicates per Treatment: 10	
Length of Test: 7 days	Number of Organisms per Replicate: 1	
Type of Food and Quantity per Chamber: 0.3 mL Alg/YTC	Feeding Frequency: Once, before organism addition	
Test Substance Characterization Parameters and Frequency:	Hardness: Initiation, Day 3, 6, termination	Alkalinity: Initiation, Day 3, 6, termination
NH_3: Initiation pH: Daily	Conductivity: Daily TDS: Daily	TRC: Initiation
Test Conc.: 0 (Simulated RW WITH DOC/Control Dilution H_2O), 4.5, 6.5, 9.2, 13.2, 18.9, 26.9, 38.5 µg/L Nickel		

Reference Toxicant Data - Mean Reproduction

Test Dates: 12/9/16 to 12/16/16	LC_{50} or [✓ IC_{25}] (Circle): 496.1 mg/L Cl^-
Hist. 95% Control Limits: 237.5 to 1179 mg/L Cl^-	Method for Determining Ref. Tox. Value: Linear Interpolation

Special Procedures and Considerations

For seeding test, use neonates from simulated effluent monoboards for the control/dilution water and the nickel exposures.

Total volumes for each concentration will be prepared on different days. Control/dilution water will have B_{12} and Se nutrients.
Days 0, 3, 6: prepare 450 mL each day.
Days 1, 2, 4, 5: prepare 400 mL each day.
Prepare each concentration in a 500-mL graduated cylinder, although you will only be preparing **400 or 450 mL** on the specific days. Fill the cylinder with ~80% dilution water, then add appropriate amount of nickel stock to achieve desired concentration, then fill to line with dilution water. Mix well. Let solutions equilibrate for 3 hours at test temperature.

ATTENTION: Please be extra careful when pipetting and filling. Acid rinse and DI rinse the graduated cylinder after each day's use. Rinse out beakers with DI very well after each day.

METALS SAMPLING SCHEDULE: New Total and Dissolved (0.45 um): Day 0, 3, 6; Old (Total and Dissolved composite): Day 4 and 7

READ PROTOCOL PRIOR TO WORKING ON THIS TEST. There will be measurements of TDS daily. Hardness/Alkalinity will be measured in multiple concentrations on multiple days (see protocol).

ES 1/17/17
ASC 2/10/17

Page:
OSU AquaTox QA Form No. 112
Revision: 0
Effective: 02/2010

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1126 CDC – WITH DOC

Chemical	Nickelous Chloride Hexahydrate $NiCl_2 \times 6H_2O$					
Chemical Manufacturer	JT Baker					
Chemical Lot #	L05582					
Nominal Stock Concentration	**20 mg/L Ni**					
Test Substance Stock Preparation Date and Time	Date: 12/15/16 @ 1100		Date: @		Date: @	
Date(s) Used	12/16/16					
	12/19/16					
	12/22/16					

PREPARATION OF TEST SOLUTIONS

Nominal Chemical Conc. (µg/L Ni)		Nominal Stock Volume (µL)	Test Stock Volume (µL)						
			Day 0	Day 3	Day 6				
#1	0 (Control WITH DOC) [1]	0	0	0	0				
#2	4.5	101.3	101	101	101				
#3	6.5	146.3	146	146	146				
#4	9.2	207	207	207	207				
#5	13.2	297	297	297	297				
#6	18.9	425.3	425	425	425				
#7	26.9	605.3	605	605	605				
#8	38.5	866.3	866	866	866				
Total		2648.5	2647	2647	2647				
Total Volume per Treatment **(450 mL)**			450	450	450				
Dilution Water ID [1]			Sim. RW with DOC RW *1474-B	Sim. RW with DOC RW *1474-B	Sim. RW with DOC RW *1474-B				
Concurrent Control Water ID [1]									
Date			12/16/16	12/19/16	12/22/16				
Time			1145	1030	1030				
Initials			ES	ES	ES				

[1] Both the concurrent control (Very Hard RW) and the 20% Diluted Simulated Effluent RW (Control/dilution water) will have nutrients: B_{12} and Se

(Stock: Vitamin B_{12} 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B_{12})

(Stock: Na_2SeO_4 120 mg/L Stock = add 60 µL/L to achieve 3 µg/L Se)

Simulated RW *: water is full strength simulated effluent diluted by 20% with deionized water. No DOC added.

ES 1/17/17

ASC 2/10/17

Page:
OSU AquaTox QA Form No. 112
Revision: 0
Effective: 02/2010

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1126 CDC – WITH DOC

Chemical	Nickelous Chloride Hexahydrate $NiCl_2 \times 6H_2O$					
Chemical Manufacturer	JT Baker					
Chemical Lot #	L05582					
Nominal Stock Concentration	**20 mg/L Ni**					
Test Substance Stock Preparation Date and Time	Date: 12/15/16 @ 1100		Date: @		Date: @	
Date(s) Used	12/17/16					
	12/18/16					
	12/20/16					
	12/21/16					

PREPARATION OF TEST SOLUTIONS

Nominal Chemical Conc. (µg/L Ni)		Nominal Stock Volume (µL)	Test Stock Volume (µL)						
			Day 1	Day 2	Day 4	Day 5			
#1	0 (Control WITH DOC) [1]	0	0	0	0	0			
#2	4.5	90	90	90	90	90			
#3	6.5	130	130	130	130	130			
#4	9.2	184	184	184	184	184			
#5	13.2	264	264	264	264	264			
#6	18.9	378	378	378	378	378			
#7	26.9	538	538	538	538	538			
#8	38.5	770	770	770	770	770			
Total		2354	2354	2354	2354	2354			
Total Volume per Treatment **(400 mL)**			400	400	400	400			
Dilution Water ID [1]			Sim. RW with DOC RW * 1474-B	Sim. RW with DOC RW * 1474-B	Sim. RW with DOC RW * 1474-B	Sim. RW with DOC RW * 1474-B			
Concurrent Control Water ID [1]			▨	▨	▨	▨			
Date			12/17/16	12/18/16	12/20/16	12/21/16			
Time			1210	1225	1150	1115			
Initials			ES	ES	TT	ES			

[1] Control/dilution water will have nutrients: B_{12} and Se

(Stock: Vitamin B_{12} 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B_{12})

(Stock: Na_2SeO_4 120 mg/L Stock = add 60 µL/L to achieve 3 µg/L Se)

Simulated RW *: water is full strength simulated effluent diluted by 20% with deionized water. WITH DOC added.

ES 1/17/17

ASC 2/10/17 ①

① ASC 2/10/17 E

Page:
OSU AquaTox QA Form No. 041
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1126 CDC – **WITH DOC**

Test Species: *C. dubia*

Conc. (**µg/L Ni**)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter # All conc.	Remarks
#1 0 (Sim w/DOC)										
pH	8.56	8.54	8.67	8.71	8.80	8.68	8.69		M16	
D.O. (mg/L)	8.4	8.3	8.9	8.8	8.6	8.6	8.6		M07	
Temp. (°C)	25	① ~~24~~ 25	25	25	25	25	25		dig	
Cond. (µS/cm)	2270	① ~~22~~ 2320	2300	2330	2340	2330	2330		M03	
TDS (mg/L)	1145	1170	1169	1178	1184	1178	1181		M03	
Hard. (mg/L)	324			304			304		titr	
Alk. (mg/L)	392			400			408		titr	
TRC (mg/L)	<0.05			NT			NT		PC II	
NH_3 (mg/L)	<1.0			NT			NT		M11	
#2 4.5										
pH	8.56	8.55	8.69	8.74	8.80	8.70	8.69			
D.O. (mg/L)	8.4	8.3	8.8	8.8	8.6	8.6	8.5			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2270	2330	2320	2320	2340	2340	2330			
TDS (mg/L)	1148	1180	1172	1176	1183	1181	1181			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
#3 6.5										
pH	8.56	8.56	8.70	8.74	8.80	8.71	8.64			
D.O. (mg/L)	8.4	8.3	8.8	8.8	8.6	8.5	8.5			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2270	2320	2330	2330	2340	2340	2330			
TDS (mg/L)	1147	1171	1177	1177	1183	1180	1176			
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L)										
NH_3 (mg/L)										
Date:	12/16/16	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16			NT = not taken
Time:	1500	1520	1540	1355	~~1555~~ ② 1455	1425	1335			
Initials:	TH	ES	ES	ASC	TH	ES	MS			

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, OSU TOX QA Form No. 011.
* Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting dilutions is assumed to also be 25°C.

① ES 12/17/16 E ② TH 12/20/16

ES 1/17/17
ASC 2/10/17

Page:
OSU TOX QA Form No. 041
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1126 CDC – **WITH DOC**

Test Species: *C. dubia*

Conc. (**µg/L Ni**)		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter # All conc.	Remarks
#4	9.2										
pH		8.56	8.57	8.70	8.74	8.81	8.72	8.69			
D.O. (mg/L)		8.3	8.2	8.7	8.8	8.6	8.5	8.4			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2270	2320	2330	2330	2340	2340	2320			
TDS (mg/L)		1148	1170	1175	1176	1183	1182	1174			
Hard. (mg/L)					304			312			
Alk. (mg/L)					404			396			
TRC (mg/L)					NT			NT			
NH_3 (mg/L)					NT			NT			
#5	13.2										
pH		8.57	8.56	8.70	8.75	8.80	8.72	8.70			
D.O. (mg/L)		8.3	8.2	8.7	8.8	8.6	8.5	8.4			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2270	2310	2330	2330	2340	2340	2320			
TDS (mg/L)		1147	1168	1175	1175	1182	1181	1172			
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
#6	18.9										
pH		8.57	8.56	8.71	8.75	8.80	8.72	8.70			
D.O. (mg/L)		8.3	8.2	8.7	8.8	8.6	8.5	8.4			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2270	2310	2330	2320	2340	2330	2320			
TDS (mg/L)		1147	1167	1175	1174	1182	1181	1174			
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
	Date:	12/16/16	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16			NT = not taken
	Time:	1500	1520	1540	1355	① ~~[illegible]~~ 1455	1425	1335			
	Initials:	TH	ES	ES	ASC	TH	ES	MJ			

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, OSU TOX QA Form No. 011.
* Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting dilutions is assumed to also be 25°C.

① TH 12/20/16 E

ES 1/17/17
ASC 2/10/17

Page:

OSU TOX QA Form No. 041

Revision: 0

Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1126 CDC – **WITH DOC**

Test Species: *C. dubia*

Conc. (µg/L Ni)		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter # All conc.	Remarks
#7	26.9										
pH		8.56	8.56	8.71	8.74	8.81	8.73	8.70			
D.O. (mg/L)		8.3	8.2	8.7	8.8	8.6	8.4	8.4			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2270	2310	2330	2310	2340	2330	2320			
TDS (mg/L)		1146	1167	1175	1170	1181	1179	1174			
Hard. (mg/L)											
Alk. (mg/L)											
TRC (mg/L)											
NH_3 (mg/L)											
#8	38.5										
pH		8.57	8.57	8.71	8.74	8.80	8.72	8.70			
D.O. (mg/L)		8.3	8.2	8.7	8.8	8.5	8.4	8.4			
Temp. (°C)		25	25	25	25	25	25	25			
Cond. (µS/cm)		2270	2310	2320	2320	2330	2330	2310			
TDS (mg/L)		1145	1168	1173	1171	1180	1178	1169			
Hard. (mg/L)					304			304			
Alk. (mg/L)					400			396			
TRC (mg/L)					NT			NT			
NH_3 (mg/L)					NT			NT			
	Date:	12/16/16	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16			NT= not taken
	Time:	1500	1520	1540	1355	1455	1425	1335			
	Initials:	TH	ES	ES	ASC	TH	ES	ms			

NOTE: Hardness, alkalinity, TRC, and NH_3 data appearing on this page have been transcribed from the wet chemistry log, OSU TOX QA Form No. 011.
* Dilution/control water and effluent were brought to 25°C prior to mixing the dilution series. The temperature of resulting dilutions is assumed to also be 25°C.

ES 1/17/17

ASC 2/10/17

OSU AquaTox QA Form No. 025
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1126 CDC – **WITH DOC**

Test Species: *C. dubia*

Conc. (µg/L Ni)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter # All Conc.	Remarks
#1 Con. DOC										
pH	8.70	8.78	8.79	8.81	8.73	8.64	8.78		M16	
D.O. (mg/L)	8.7	8.9	9.0	8.8	8.9	8.8	8.6		M07	
Cond. (µS/cm)	2370	2470	2370	2410	2570	2440	2440		M03	
TDS (mg/L)	1198	1243	1200	1221	1308	1237	~~2440~~ 1265		M03	
Temp. (°C)	24	25	25	25	25	25	25		Dig.	
#2 4.5										
pH	8.70	8.77	8.80	8.80	8.74	8.65	8.79			
D.O. (mg/L)	8.5	8.9	9.0	8.6	8.8	8.8	8.5			
Cond. (µS/cm)	2410	2470	2750	2430	2630	2710	2840			
TDS (mg/L)	1217	1333	1399	1228	1337	1374	~~2840~~ 1448			
Temp. (°C)	24	25	25	25	25	25	25			
#3 6.5										
pH	8.71	8.80	8.79	8.82	8.75	8.66	8.78			
D.O. (mg/L)	8.6	9.0	8.9	8.6	8.9	8.8	8.6			
Cond. (µS/cm)	2540	2510	2390	2460	2530	2520	2560			
TDS (mg/L)	1288	1253	1211	1247	1295	1292	1302			
Temp. (°C)	24	25	25	25	25	25	25			
#4 9.2										
pH	8.73	8.81	8.80	8.83	8.74	8.67	8.78			
D.O. (mg/L)	8.6	8.9	8.9	8.7	8.9	8.9	8.6			
Cond. (µS/cm)	2650	2600	2430	2550	2730	2660	2560			
TDS (mg/L)	1343	1321	1231	1300	1392	1350	1301			
Temp. (°C)	24	25	25	25	25	25	25			
#5 13.2										
pH	8.71	8.79	8.79	8.82	8.75	8.67	8.78			
D.O. (mg/L)	8.6	9.0	8.9	8.7	8.9	8.8	8.5			
Cond. (µS/cm)	2430	2460	2380	2450	2570	2490	2560			
TDS (mg/L)	1233	1239	1203	1260	1306	1263	1300			
Temp. (°C)	24	25	25	25	25	25	25			
#6 18.9										
pH	8.69	8.78	8.79	8.81	8.75	8.68	8.79			
D.O. (mg/L)	8.5	8.9	8.8	8.6	8.9	8.8	8.5			
Cond. (µS/cm)	2360	2450	2420	2370	2590	2440	2510			
TDS (mg/L)	1193	1238	1227	1199	1314	1233	1271			
Temp. (°C)	24	25	25	25	25	25	25			
#7 26.9										
pH	8.72	8.81	8.80	8.83	8.75	8.67	8.79			
D.O. (mg/L)	8.5	9.0	8.9	8.7	8.9	8.9	8.5			
Cond. (µS/cm)	2650	2560	2410	2540	2580	2570	2590			
TDS (mg/L)	1349	1299	1220	1293	1320	1305	1318			
Temp. (°C)	24	25	25	25	25	25	25			
Date:	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16			
Time:	1600	1620	1435	1510	1520	1455	1420			
Initials:	ES	ES	ASC	ES	MS	MS	TH			

ASC 2/10/17 ES 1/17/17

OSU AquaTox QA Form No. 025
Revision: 0
Effective: 02/2010

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1126 CDC – **WITH DOC**

Test Species: *C. dubia*

Conc. (µg/L Ni)		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter # All Conc.	Remarks
#8	38.5										
pH		8.70	8.79	8.81	8.81	8.75	8.69	8.79			
D.O. (mg/L)		8.5	8.9	8.9	8.6	8.9	8.7	8.7			
Cond. (µS/cm)		2370	2500	2550	2380	2610	2520	2390			
TDS (mg/L)		1198	① 1~~3~~267	1293	1203	1326	1277	② ~~1318~~ 1210			
Temp. (°C)		24	25	25	25	25	25	25			
Date:		12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16			
Time:		1600	1620	1435	1510	1520	1455	1420			
Initials:		ES	ES	ASC	ES	MJ	MJ	TH			

① ES 12/18/16 E ② TH 12/23/16 E

ASC 2/10/17 ES 1/17/17

SUBJECT: *CERIODAPHNIA DUBIA* CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1126 CDC – **WITH DOC**

		Number of Neonates Produced and Survival of Original Organisms												
Conc. µg/L Ni	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
Con. (w/DOC)	1	0	0	0	0	0	0	0	0	0	0			
#1	2	0	0	0	0	0	0	0	0	0	0			
	3	1/4	0	0	0	0	0	0	0	0	0			
	4	0	1/7	1/6	1/8	1/6	1/8	1/5	1/6	1/4	1/7			
	5	2/8	2/14	2/14	2/13	0	2/15	2/8	2/12	2/14	2/15			
	6	3/21	0	0	0	2/12	0	0	0	0	0			
	7	4/①21	3/23	3/17	3/18	3/22	3/24	3/18	3/22	3/21	3/22			
	8	4th brood not added												
Total		33	44	37	39	40	47	31	40	39	44			
4.5	1	0	0	0	0	0	0	0	0	0	0			
#2	2	0	0	0	0X	0	0	0	0	0	0			
	3	0	0	0	↓	0	0	0	0	0	0			
	4	1/6	1/7	1/6		1/7	1/8	1/8	1/6	1/5	1/5			
	5	2/11	2/12	0		2/12	2/14	0	2/14	2/18	2/18			
	6	0	2/2	2/12		0	0	2/14	0	0	0			
	7	3/24	3/23	3/20		3/19	3/20	3/20	3/20	3/20	3/20			
	8													
Total		41	44	38	0	38	42	42	40	43	43			
6.5	1	0	0	0	0	0	0	0	0	0	0			
#3	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/7	1/7	1/9	1/7	1/6	1/6	1/8	1/6	1/7	1/7			
	5	2/13	2/12	2/13	1/1	0	2/12	2/10	0	0	2/16			
	6	0	2/2	0	2/13	2/4X	0	0	2/13	2/14	0			
	7	3/23	3/20	3/23	3/22	↓	3/24	3/20	3/19	3/16	3/17			
	8													
Total		43	41	45	43	10	42	38	38	37	40			

DAY:	1	2	3	4	5	6	7	8
Date:	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16	
Time:	1530	1600	1400	1530	1500	1420	1420	
Initials:	ES	ES	ES	ES	MS	ES	MS	

Key to Symbols:
X = Original organism has died.
M = Male.

① MS 12/23/16 E

ASC 2/10/17 ES 1/17/17

Page:
OSU AquaTox QA Form No. 024
Revision: 0
Effective: 2/2010

SUBJECT: *CERIODAPHNIA DUBIA* CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1126 CDC – **WITH DOC**

		Number of Neonates Produced and Survival of Original Organisms												
Conc. µg/L Ni	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
9.2	1	0	0	0	0	0	0	0	0	0	0			
#4	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/6	1/6	1/8	1/6	1/4	1/6	1/4	1/6	1/8	1/6			
	5	2/12	2/12	2/14	2/15	1/1	2/13	0	2/14	2/11	2/13			
	6	0	0	0	0	2/14	0	2/17	0	0	0			
	7	3/21	3/~~23~~ 15 ①	3/19	3/~~16~~ 14 ①	3/~~22~~ 18 ①	3/~~24~~ 20 ①	3/18	3/~~22~~ 16 ①	3/~~21~~ 19 ①	3/~~22~~ 19 ①			
	8													
	Total	39	33	41	35	37	39	39	36	38	38			
13.2	1	0	0	0	0	0	0	0	0	0	0			
#5	2	0	0	0	0	0	0	0	0	0	0			
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/6	1/5	1/5	1/6	1/6	1/5	1/6	1/5	1/5	1/7			
	5	2/10	2/13	2/14	0	0	2/13	2/12	2/10	2/14	2/10			
	6	0	0	0	2/13	2/14	0	0	0	0	0			
	7	3/16	~~3/23~~ 3/20 ①	3/~~20~~ 18 ①	3/18	3/22	3/17	3/15	3/14	3/26	3/18			
	8													
	Total	32	38	37	37	42	35	33	29	39	35			
18.9	1	0	0	0	0	0	0	0	0	0	0			
#6	2	0	0	0	0	0	0	0	0	0	0			
	3	1/6	0	0	0	0	0	0	0	0	0			
	4	0	1/5	1/6	1/4	1/5	1/6	1/6	1/7	1/5	1/6			
	5	2/8	0	2/14	0	0	0	0	0	0	0			
	6	3/11	2/8	0	2/12	2/12	2/10	2/11	2/8	2/13	2/10			
	7	0	0	3/16	0	3/20	3/20	3/12	3/12	3/20	3/12			
	8													
	Total	25	13	36	16	37	36	29	27	38	28			

DAY:	1	2	3	4	5	6	7	8	
Date:	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16		
Time:	1530	1600	1400	1530	1500	1420	1420		
Initials:	ES	ES	ES	ES	MJ	ES	MJ		

Key to Symbols:
X = Original organism has died.
M = Male.

① MJ 12/23/16 E

ASC 2/10/17 ES 1/17/17

SUBJECT: *CERIODAPHNIA DUBIA* CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1126 CDC – **WITH DOC**

Conc. µg/L Ni	Day	A	B	C	D	E	F	G	H	I	J	Total	Mean	Remarks
		Number of Neonates Produced and Survival of Original Organisms												
26.9	1	0	0	0	0	0	0	0	0	0	0			Ⓑ org is pale
#7	2	0	0	0	0	0	0	0	0	0	0			12/20/16 12/21/16 ms
	3	0	0	0	0	0	0	0	0	0	0			
	4	1/6 Ⓑ①	1/4	1/6 Ⓑ①	1/5	1/5 Ⓑ①	1/6	1/5	1/5	1/3 Ⓑ①	1/8			
	5	2/5	2/6	0	2/10	0	0	0	2/8	0	0			
	6	0	0	2/11	0	2/14	2/11	2/10	0	2/4	2/5			
	7	0	3/10	0	3/17	0	3/4	3/14	3/8	0	0 X			
	8										↓			
Total		11	20	17	32	19	21	29	21	7	13			
38.5	1	0	0	0	0 X Ⓐ	0	0	0	0	0	0			Ⓐ org not found
#8	2	0	0	0		0	0 X	0	0	0	0			12/17/16 ES
	3	0	0	0		0 Ⓑ①		0	0	0	0			
	4	1/4 Ⓑ	1/4 X	1/6 Ⓑ		1/5 Ⓑ		1/6	1/6	1/5 Ⓑ	1/6			
	5	1/1 ②		0 ②		0 ~~Ⓑ~~		0	0	0 B	0			
	6	0 X		2/2 X		2/10		2/9	2/12	0 X	2/6 X			
	7					0		3/7	3/2					
	8	↓	↓	↓	↓		↓			↓	↓			
Total		5	4	8	–	15	0	22	20	5	12			
Total														

DAY:	1	2	3	4	5	6	7	8	
Date:	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16		
Time:	1530	1600	1400	1530	1500	1420	1420		
Initials:	ES	ES	ES	ES/TH	MS	ES	MS		

Key to Symbols:

X = Original organism has died.

M = Male.

① TH 12/20/16 E

② MS 12/21/16 E

ASC 2/10/17 ES 1/17/17

Page:
OSU AquaTox QA Form No. 026
Revision: 0
Effective: 02/2010

SUBJECT: DAILY TOXICITY TEST LOG

Project Number: Ni WER 1126 CDC – **WITH DOC**

Test Species: *C. dubia*

General Comments	For Diluted Simulated Effluent RW: Neonates obtained from (culture): CD 120816 Sim. Eff. on 12/16/16 MS	Feeding
Test Day 0 12/16/16	3-hr Equilibrium Started at: 1145 ES 3-hr Equilibrium Ended at: 1445 MS Test Organisms Added at: 1500 MS Checked by: TH Total and Dissolved (0.45 μm) sampled at: 1510 TH	0.3 mL Alg/YTC @ 1450 MS
Test Day 1 12/17/16	3-hr Equilibrium Started at: 1210 ES 3-hr Equilibrium Ended at: 1510 ES	0.3 mL Alg/YTC @ 1530 ES
Test Day 2 12/18/16	3-hr Equilibrium Started at: 1225 ES 3-hr Equilibrium Ended at: 1530 ES	0.3 mL Alg/YTC @ 1600 ES
Test Day 3 12/19/16	3-hr Equilibrium Started at: 1030 ES 3-hr Equilibrium Ended at: 1345 ES New Total and Dissolved (0.45 μm) sampled at: 1440 ES 1-L control sampled @ 1445 ASC	0.3 mL Alg/YTC @ 1400 ES
Test Day 4 12/20/16	3-hr Equilibrium Started at: 1150 TH 3-hr Equilibrium Ended at: 1450 ES Old (comp) Total and Dissolved (0.45 μm) sampled at: 1545 ES	0.3 mL Alg/YTC @ 1500 ES
Test Day 5 12/21/16	3-hr Equilibrium Started at: 1115 ES 3-hr Equilibrium Ended at: 1420 ES	0.3 mL Alg/YTC @ 1430 ES
Test Day 6 12/22/16	3-hr Equilibrium Started at: 1030 ES 3-hr Equilibrium Ended at: 1330 ES New Total and Dissolved (0.45 μm) sampled at: 1420 MS	0.3 mL Alg/YTC @ 1420 ES
Test Day 7 12/23/16	Test Taken down at: 1430 TH Old (comp) Total and Dissolved (0.45 μm) sampled at: 1500 TH	

ASC 2/10/17 ES 1/17/17

SIMULATED EFFLUENT FOR TESTING - *Ceriodaphnia dubia*

Total hardness = 400 mg/L as $CaCO_3$
Alkalinity = 400 mg/L as $CaCO_3$
Volume of water = 44 L (with 20% addition, will be total volume of 55-L)

Amount weighed		
12.47	12.4657	grams $CaSO_4$ $2H_2O$
12.45	12.4523	grams $MgSO_4$
8.57	8.5712	grams KCl
23.98	23.9800	grams NaCl
40.66	40.6560	grams $NaHCO_3$

Estimated/Calculated Nominal (mg/L)	
52.2	Ca
56.5	Mg
467.3	Na
102.17	K
423.5	Cl
348.5	SO_4
488.1	HCO_3

	Manufacturer	Lot #
$CaSO_4 \cdot 2H_2O$	ACROS Organics	A0363568
$MgSO_4$	EMD	151020001
KCl	Alfa Aesar	E29Y012
NaCl	EMD	YC04E/16C100004
$NaHCO_3$	MaCRON	0000134421

Recon. Water #: 1474

Test #: Ni WER 1125 CDC, Ni WER 1126 CDC

Date/Time Prepared: Start mixing 12/12/16 @1600

Technician: TH

PREPARATION STEPS:

✓ 1) In 4-Liter Flask, add $CaSO_4 \cdot 2H_2O$ to 3-L DI. Put on stir plate. Mix overnight

✓ 2) In 4-Liter Flask, add $MgSO_4$ to 3-L DI. Put on stir plate. Mix overnight

✓ 3) In 2-Liter Beaker, add KCl, NaCl, and $NaHCO_3$ to 2-L DI. Put on stir plate. Mix overnight

✓ 4) After the 3 containers have mixed overnight, combine and add 36 Liters DI for a total of 44 Liters in a 55-L carboy.

✓ 5) Stir very well after combining. Put airstone (clean stone with clean tubing with a stopper to weigh it down) and bubble CO2 until pH is below 6.0 (preferably 5.6 - 5.8).

✓ 6) Flush headspace in carboy with CO2 and seal top and allow to sit overnight.

✓ 7) The next day, bubble air to bring pH up. After pH is above 8.0, add 11-L DI and mix well. This will be the "diluted simulated effluent". Measure out 27 Liters into separate carboy.

8) To one carboy of 27 Liters, add 787.5 mg DOC (Suwannee River: 14 mg/L DOC at a 48% composition).

✓ 9) Aerate each carboy lightly overnight for use the next day.

ASC 2/10/17 ES 1/26/17

SUPPLEMENTAL DATA:
Additional Statistical Analysis
Nickel Water-Effect Ratio (WER) Toxicity Test Data

Prepared for

SANITARY DISTRICT OF DECATUR
501 Dipper Lane
Decatur, IL 62522
USA

Prepared by

OREGON STATE UNIVERSITY
DEPARTMENT OF ENVIRONMENTAL AND MOLECULAR TOXICOLOGY
AQUATIC TOXICOLOGY LABORATORY
33972 Texas St. SW
Albany, OR 97321
USA
T. 541.926.1254

11 August 2017

This supplemental data includes additional exposure-effects analysis of toxicity test data for the following studies: Ni Sim 1008 CDC (OSU 2016) and Ni WER 1126 CDC (OSU 2017) and Ni WER 1132R CDC (OSU 2017).

Survival and reproductive endpoints were originally reported as mean survival weighted by standard deviation and mean reproduction of original female weighted by standard deviation analyzed. Both endpoints were analyzed by threshold sigmoid regression analysis. The additional analyses of the data are reported in Table 1 and included as raw statistical data attachments. The analysis includes the following endpoints, as determined by the TRAP statistical program:

- Mean survival by tolerance distribution
- Individual replicate reproduction of original female unweighted
- Individual replicate reproduction of original female weighted by standard deviation of treatment
- Mean reproduction of surviving females weighted by standard deviation
- Individual replicate reproduction of surviving female unweighted
- Individual replicate reproduction of surviving female weighted by standard deviation of treatment

The results (Table 1) demonstrate that, even with additional analyses, actual effect concentrations showed little change and did not depend upon the details of the analysis.

REFERENCES

Oregon State University Aquatic Toxicology Laboratory. 2016. Water-Effect Ratio (WER) Testing: Chronic toxicity of a nickel spiked simulated effluent and a nickel spiked whole effluent to the cladoceran, *Ceriodaphnia dubia*. Prepared for the Sanitary District of Decatur. Final Report: 03 June 2016. Test #: Ni Sim 1008 CDC.

Oregon State University Aquatic Toxicology Laboratory. 2017. Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*. Prepared for the Sanitary District of Decatur. Final Report: 29 June 2017. Test #s: Ni WER 1126 CDC and Ni WER 1132R CDC.

Toxicity Relationship Analysis Program (TRAP). U.S. Environmental Protection Agency. National Health and Environmental Effects Research Laboratory, Duluth, MN. Version 1.30.

Table 1. Additional statistical analysis of Nickel WER toxicity test data

Endpoint		**LC20/EC20**	**95% CIs**	**LC20/EC20**	**95% CIs**	**LC20/EC20**	**95% CIs**
		Test ID: Ni Sim 1008 CDC (without added DOC)		**Test ID: Ni WER 1132R CDC (without added DOC)**		**Test ID: Ni WER 1126 CDC (with added DOC)**	
Survival	**Mean ***	13.0	11.8 – 14.3	8.3	7.3 – 9.4	26.3	23.6 – 29.3
	Mean – Tolerance Distribution	13.6	10.7 – 17.1	9.7	7.8 – 12.0	26.4	21.5 – 32.5
Repro/ Original	**Mean ***	7.4	5.2 – 10.5	8.0	6.1 – 10.6	16.1	14.6 – 17.7
	Replicates unweighted	7.7	6.1 – 9.9	6.8	5.4 – 8.7	16.1	13.3 – 19.4
	Replicates weighted	7.4	5.9 – 9.3	7.9	6.6 – 9.6	16.1	14.0 – 18.6
Repro/ Surviving	**Mean**	8.3	5.6 – 12.3	8.9	5.8 – 13.6	14.2	10.6 – 19.2
	Replicates unweighted	7.8	6.1 – 10.1	7.1	5.8 – 8.7	14.0	11.7 – 16.9
	Replicates weighted	7.5	5.9 – 9.5	8.9	7.4 – 10.8	14.1	11.9 – 16.7

* Originally reported in OSU (2016) and OSU (2017). Other values represent additional analysis.

Project: Chronic toxicity of a nickel-spiked simulated effluent to the cladoceran, *Ceriodaphnia dubia*

Sponsor: Sanitary District of Decatur
Oregon State University Aquatic Toxicology Lab
Supplemental: Statistical Re-Analysis Exercise

Nickel WER (simulated effluent tested concurrently with SDD effluent
Test ID: Ni Sim 1008 CDC

					Per original female		Per surviving female	
Nominal Treatment µg/L Ni	**Measured (Average) Dissolved Ni µg/L**	**Rep**	**Survival**	**total # of neonates**	**Average**	**Std Dev**	**Average**	**Std Dev**
0	0.7	A	1	19	24.4	3.1	24.4	3.1
0	0.7	B	1	24				
0	0.7	C	1	23				
0	0.7	D	1	25				
0	0.7	E	1	22				
0	0.7	F	1	23				
0	0.7	G	1	25				
0	0.7	H	1	30				
0	0.7	I	1	25				
0	0.7	J	1	28				
4.9	4.7	A	1	23	22.3	1.4	22.3	1.4
4.9	4.7	B	1	24				
4.9	4.7	C	1	21				
4.9	4.7	D	1	23				
4.9	4.7	E	1	22				
4.9	4.7	F	1	21				
4.9	4.7	G	1	22				
4.9	4.7	H	1	25				
4.9	4.7	I	1	21				
4.9	4.7	J	1	21				
7	6.4	A	1	18	19.9	4.1	19.9	4.1
7	6.4	B	1	26				
7	6.4	C	1	21				
7	6.4	D	1	21				
7	6.4	E	1	17				
7	6.4	F	1	19				
7	6.4	G	1	25				
7	6.4	H	1	22				
7	6.4	I	1	12				
7	6.4	J	1	18				
10	9.3	A	1	12	18.3	3.8	18.3	3.8
10	9.3	B	1	20				
10	9.3	C	1	21				
10	9.3	D	1	15				
10	9.3	E	1	18				
10	9.3	F	1	19				
10	9.3	G	1	16				
10	9.3	H	1	24				
10	9.3	I	1	23				
10	9.3	J	1	15				

ASC 8/2/17

Project: Chronic toxicity of a nickel-spiked simulated effluent to the cladoceran, *Ceriodaphnia dubia*

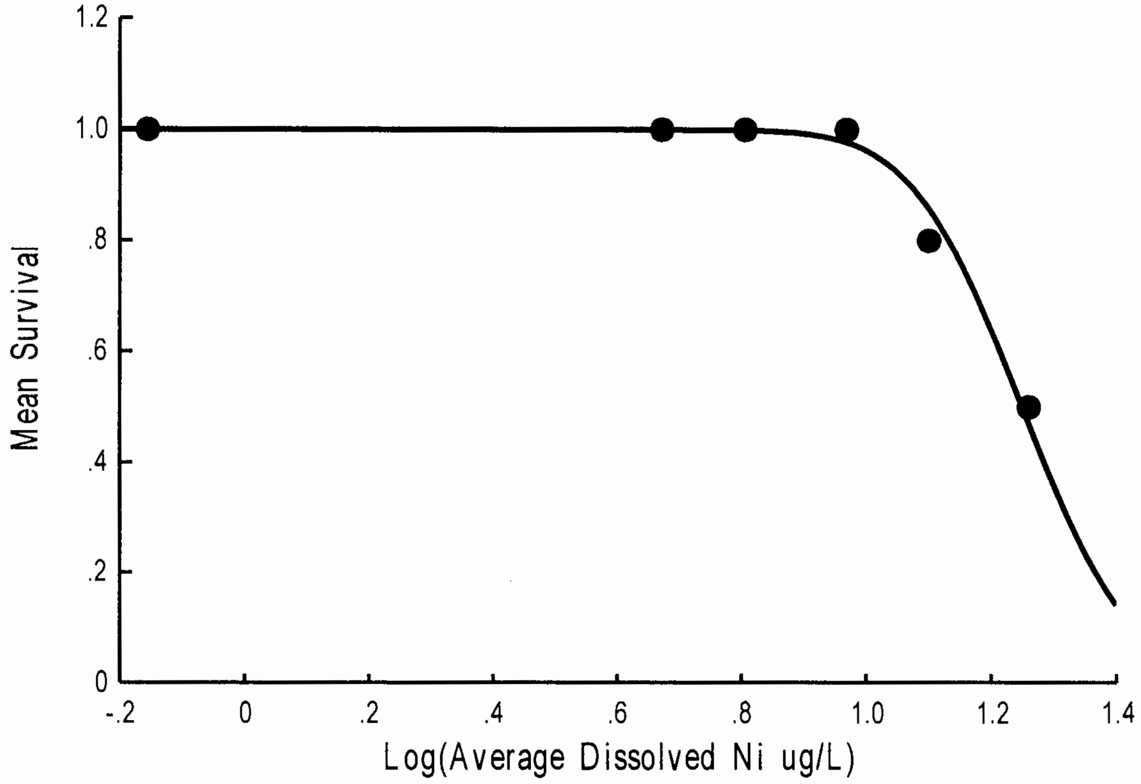
Sponsor: Sanitary District of Decatur
Oregon State University Aquatic Toxicology Lab
Supplemental: Statistical Re-Analysis Exercise

Nickel WER (simulated effluent tested concurrently with SDD effluent
Test ID: Ni Sim 1008 CDC

					Per original female		Per surviving female	
Nominal Treatment µg/L Ni	**Measured (Average) Dissolved Ni µg/L**	**Rep**	**Survival**	**total # of neonates**	**Average**	**Std Dev**	**Average**	**Std Dev**
14.3	12.6	A	1	17	12.9	5.5	15.0	3.4
14.3	12.6	B	1	20				
14.3	12.6	C	1	17				
14.3	12.6	D	**0**	**2**				
14.3	12.6	E	1	13				
14.3	12.6	F	**0**	**7**				
14.3	12.6	G	1	9				
14.3	12.6	H	1	14				
14.3	12.6	I	1	13				
14.3	12.6	J	1	17				
20.4	18.2	A	1	12	6.7	4.0	7.4	4.6
20.4	18.2	B	**0**	**9**				
20.4	18.2	C	**0**	**9**				
20.4	18.2	D	**0**	**0**				
20.4	18.2	E	1	3				
20.4	18.2	F	1	2				
20.4	18.2	G	1	9				
20.4	18.2	H	**0**	**7**				
20.4	18.2	I	1	11				
20.4	18.2	J	**0**	**5**				

ASC 8/2/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)



Parameter Summary (Gaussian Tolerance Distribution Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.2431	1.2431	0.0506	1.1366	1.3620
StdDev	0.13652	0.13652	0.05352	0.09740	0.24464
Y0	1.0000	1.0000	0.0023	0.8268	1.0000

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	17.754	13.695	23.015
20.0	13.551	10.739	17.099
10.0	11.766	8.530	16.230
5.0	10.471	6.737	16.275

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MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Data Summary

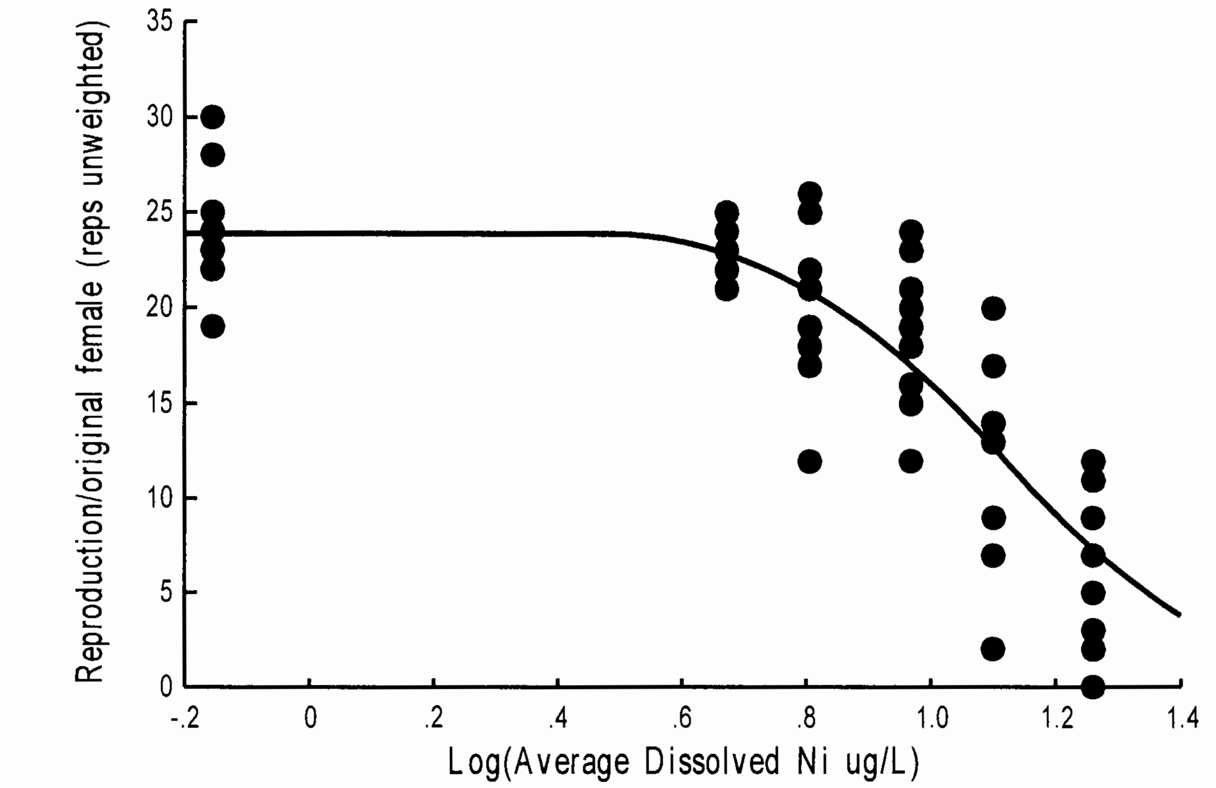
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Total N
-0.1549	1.0000	0.9999	-0.0001	10.
0.6721	1.0000	0.9999	-0.0001	10.
0.8062	1.0000	0.9992	-0.0008	10.
0.9685	1.0000	0.9779	-0.0221	10.
1.1004	0.8000	0.8572	0.0572	10.
1.2601	0.5000	0.4692	-0.0308	10.

Error Summary

No Errors

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.1000	1.1000	0.0286	1.0652	1.1797
S	1.4000	1.4000	0.2633	1.0443	2.0988
Y0	25.00	25.00	1.11	21.64	26.09

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	13.258	11.620	15.127
20.0	7.738	6.052	9.893
10.0	5.898	4.195	8.294
5.0	4.868	3.194	7.421
0.0	3.063	1.575	5.957

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	59	2949.	49.98		
Regression	2	2112.	1056.20	72.0	0.0000
Error	57	836.	14.67		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
-0.1549	30.0000	23.8630	-6.1370	1.
-0.1549	28.0000	23.8630	-4.1370	1.
-0.1549	25.0000	23.8630	-1.1370	1.
-0.1549	25.0000	23.8630	-1.1370	1.
-0.1549	25.0000	23.8630	-1.1370	1.
-0.1549	24.0000	23.8630	-0.1370	1.
-0.1549	23.0000	23.8630	0.8630	1.
-0.1549	23.0000	23.8630	0.8630	1.
-0.1549	22.0000	23.8630	1.8630	1.
-0.1549	19.0000	23.8630	4.8630	1.
0.6721	25.0000	22.8443	-2.1557	1.
0.6721	24.0000	22.8443	-1.1557	1.
0.6721	23.0000	22.8443	-0.1557	1.
0.6721	23.0000	22.8443	-0.1557	1.
0.6721	22.0000	22.8443	0.8443	1.
0.6721	22.0000	22.8443	0.8443	1.
0.6721	21.0000	22.8443	1.8443	1.
0.6721	21.0000	22.8443	1.8443	1.
0.6721	21.0000	22.8443	1.8443	1.
0.6721	21.0000	22.8443	1.8443	1.
0.8062	26.0000	20.8452	-5.1548	1.
0.8062	25.0000	20.8452	-4.1548	1.
0.8062	22.0000	20.8452	-1.1548	1.
0.8062	21.0000	20.8452	-0.1548	1.
0.8062	21.0000	20.8452	-0.1548	1.
0.8062	19.0000	20.8452	1.8452	1.

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8062	18.0000	20.8452	2.8452	1.
0.8062	18.0000	20.8452	2.8452	1.
0.8062	17.0000	20.8452	3.8452	1.
0.8062	12.0000	20.8452	8.8452	1.
0.9685	24.0000	17.0079	-6.9921	1.
0.9685	23.0000	17.0079	-5.9921	1.
0.9685	21.0000	17.0079	-3.9921	1.
0.9685	20.0000	17.0079	-2.9921	1.
0.9685	19.0000	17.0079	-1.9921	1.
0.9685	18.0000	17.0079	-0.9921	1.
0.9685	16.0000	17.0079	1.0079	1.
0.9685	15.0000	17.0079	2.0079	1.
0.9685	15.0000	17.0079	2.0079	1.
0.9685	12.0000	17.0079	5.0079	1.
1.1004	20.0000	12.7463	-7.2537	1.
1.1004	17.0000	12.7463	-4.2537	1.
1.1004	17.0000	12.7463	-4.2537	1.
1.1004	17.0000	12.7463	-4.2537	1.
1.1004	14.0000	12.7463	-1.2537	1.
1.1004	13.0000	12.7463	-0.2537	1.
1.1004	13.0000	12.7463	-0.2537	1.
1.1004	9.0000	12.7463	3.7463	1.
1.1004	7.0000	12.7463	5.7463	1.
1.1004	2.0000	12.7463	10.7463	1.
1.2601	12.0000	7.3295	-4.6705	1.
1.2601	11.0000	7.3295	-3.6705	1.
1.2601	9.0000	7.3295	-1.6705	1.
1.2601	9.0000	7.3295	-1.6705	1.
1.2601	9.0000	7.3295	-1.6705	1.
1.2601	7.0000	7.3295	0.3295	1.
1.2601	5.0000	7.3295	2.3295	1.
1.2601	3.0000	7.3295	4.3295	1.
1.2601	2.0000	7.3295	5.3295	1.
1.2601	0.0000	7.3295	7.3295	1.

ASC 8/1/17

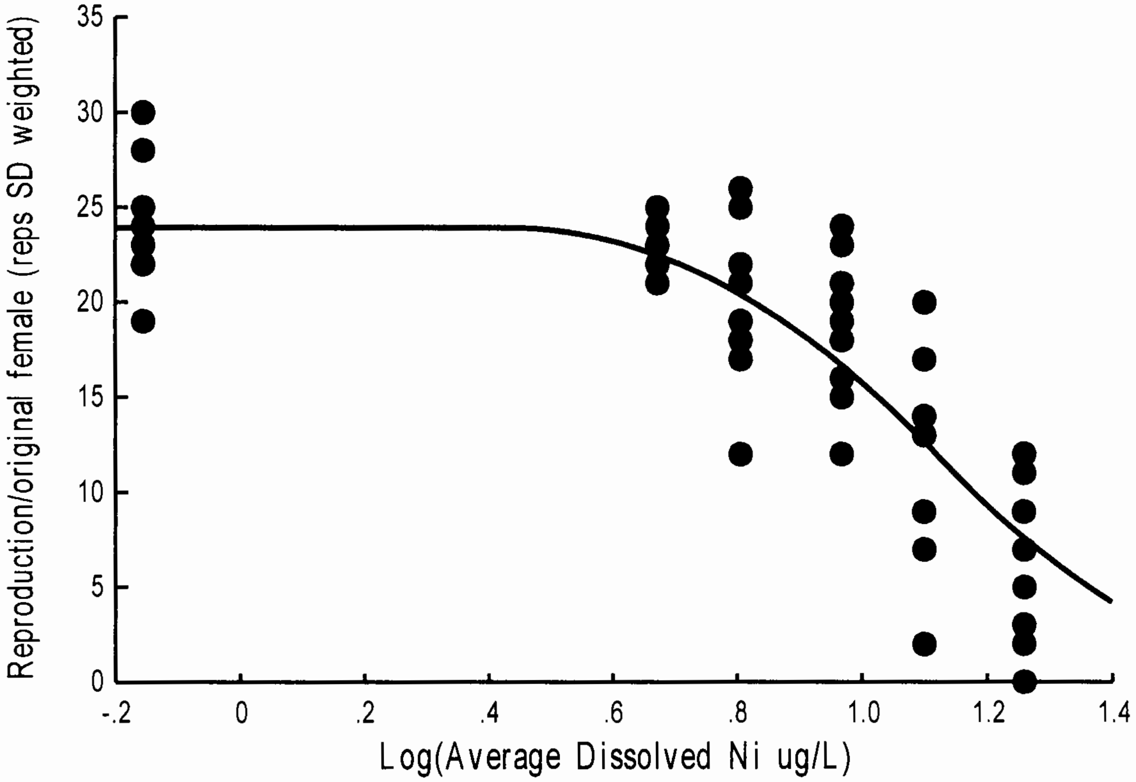
WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Error Summary

No Errors

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.1114	1.1114	0.0314	1.0582	1.1841
S	1.3645	1.3645	0.2067	1.0416	1.8694
Y0	24.40	24.40	0.91	22.09	25.74

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	13.217	11.434	15.279
20.0	7.390	5.892	9.267
10.0	5.513	4.077	7.454
5.0	4.481	3.108	6.460
0.0	2.717	1.554	4.750

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MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	59	13651.5	231.381		
Regression	2	13594.4	6797.219	6794.	0.0000
Error	57	57.0	1.000		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
-0.1549	30.0000	23.9188	-6.0812	3.1000
-0.1549	28.0000	23.9188	-4.0812	3.1000
-0.1549	25.0000	23.9188	-1.0812	3.1000
-0.1549	25.0000	23.9188	-1.0812	3.1000
-0.1549	25.0000	23.9188	-1.0812	3.1000
-0.1549	24.0000	23.9188	-0.0812	3.1000
-0.1549	23.0000	23.9188	0.9188	3.1000
-0.1549	23.0000	23.9188	0.9188	3.1000
-0.1549	22.0000	23.9188	1.9188	3.1000
-0.1549	19.0000	23.9188	4.9188	3.1000
0.6721	25.0000	22.4836	-2.5164	1.4000
0.6721	24.0000	22.4836	-1.5164	1.4000
0.6721	23.0000	22.4836	-0.5164	1.4000
0.6721	23.0000	22.4836	-0.5164	1.4000
0.6721	22.0000	22.4836	0.4836	1.4000
0.6721	22.0000	22.4836	0.4836	1.4000
0.6721	21.0000	22.4836	1.4836	1.4000
0.6721	21.0000	22.4836	1.4836	1.4000
0.6721	21.0000	22.4836	1.4836	1.4000
0.6721	21.0000	22.4836	1.4836	1.4000
0.8062	26.0000	20.4111	-5.5889	4.1000
0.8062	25.0000	20.4111	-4.5889	4.1000
0.8062	22.0000	20.4111	-1.5889	4.1000
0.8062	21.0000	20.4111	-0.5889	4.1000
0.8062	21.0000	20.4111	-0.5889	4.1000
0.8062	19.0000	20.4111	1.4111	4.1000

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8062	18.0000	20.4111	2.4111	4.1000
0.8062	18.0000	20.4111	2.4111	4.1000
0.8062	17.0000	20.4111	3.4111	4.1000
0.8062	12.0000	20.4111	8.4111	4.1000
0.9685	24.0000	16.6836	-7.3164	3.8000
0.9685	23.0000	16.6836	-6.3164	3.8000
0.9685	21.0000	16.6836	-4.3164	3.8000
0.9685	20.0000	16.6836	-3.3164	3.8000
0.9685	19.0000	16.6836	-2.3164	3.8000
0.9685	18.0000	16.6836	-1.3164	3.8000
0.9685	16.0000	16.6836	0.6836	3.8000
0.9685	15.0000	16.6836	1.6836	3.8000
0.9685	15.0000	16.6836	1.6836	3.8000
0.9685	12.0000	16.6836	4.6836	3.8000
1.1004	20.0000	12.6717	-7.3283	5.5000
1.1004	17.0000	12.6717	-4.3283	5.5000
1.1004	17.0000	12.6717	-4.3283	5.5000
1.1004	17.0000	12.6717	-4.3283	5.5000
1.1004	14.0000	12.6717	-1.3283	5.5000
1.1004	13.0000	12.6717	-0.3283	5.5000
1.1004	13.0000	12.6717	-0.3283	5.5000
1.1004	9.0000	12.6717	3.6717	5.5000
1.1004	7.0000	12.6717	5.6717	5.5000
1.1004	2.0000	12.6717	10.6717	5.5000
1.2601	12.0000	7.6118	-4.3882	4.0000
1.2601	11.0000	7.6118	-3.3882	4.0000
1.2601	9.0000	7.6118	-1.3882	4.0000
1.2601	9.0000	7.6118	-1.3882	4.0000
1.2601	9.0000	7.6118	-1.3882	4.0000
1.2601	7.0000	7.6118	0.6118	4.0000
1.2601	5.0000	7.6118	2.6118	4.0000
1.2601	3.0000	7.6118	4.6118	4.0000
1.2601	2.0000	7.6118	5.6118	4.0000
1.2601	0.0000	7.6118	7.6118	4.0000

ASC 8/1/17

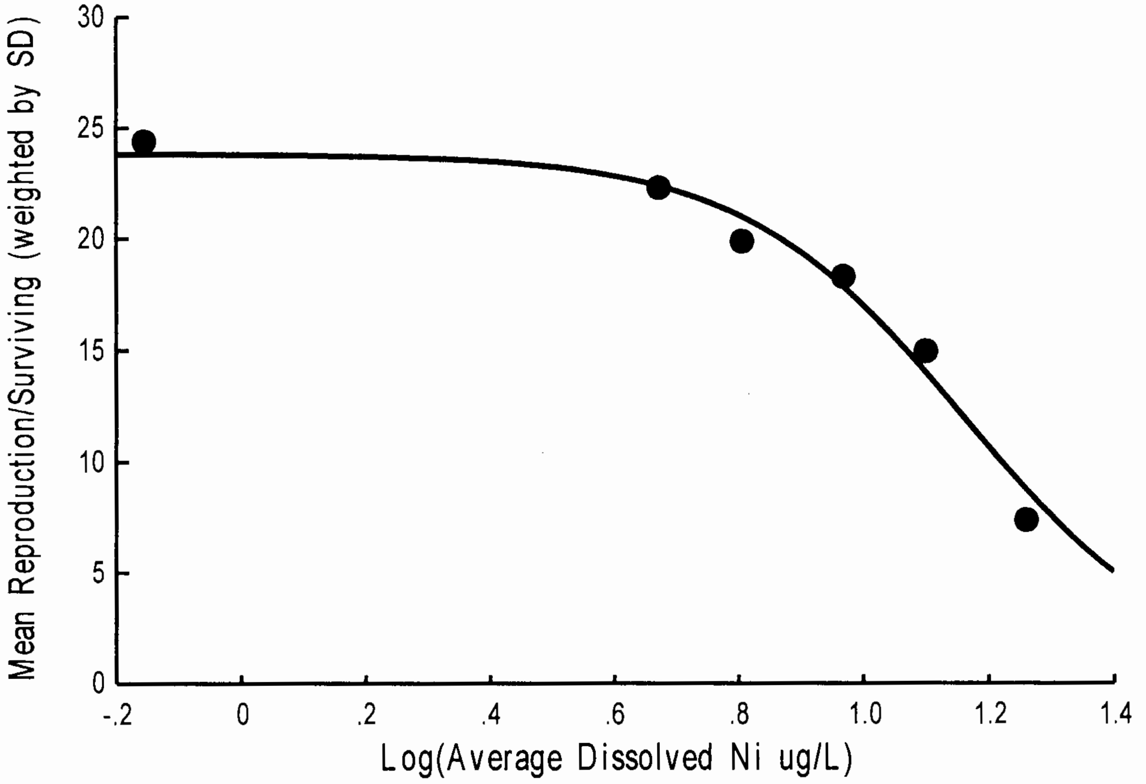
WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Error Summary

No Errors

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.1434	1.1434	0.0280	1.0762	1.2542
S	1.2597	1.2597	0.2699	0.5397	2.2578
Y0	24.40	24.40	0.82	21.20	26.44

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	14.628	11.918	17.955
20.0	8.268	5.566	12.284
10.0	5.922	3.312	10.589
5.0	4.354	2.035	9.318

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	5	1294.782	258.9563		
Regression	2	1294.481	647.2404	6453.	0.0000
Error	3	0.301	0.1003		

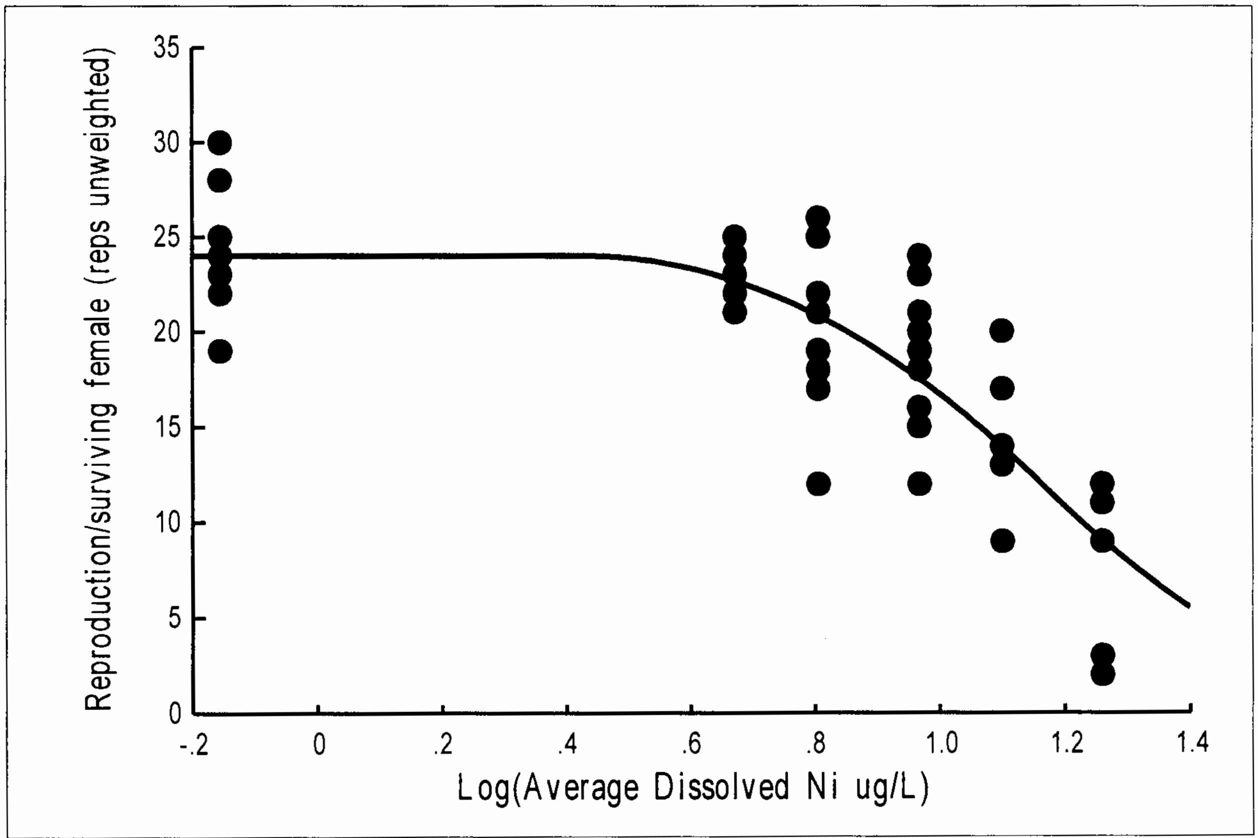
Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
-0.1549	24.4000	23.8077	-0.5923	3.1000
0.6721	22.3000	22.4029	0.1029	1.4000
0.8062	19.9000	21.0044	1.1044	4.1000
0.9685	18.3000	17.8758	-0.4242	3.8000
1.1004	15.0000	14.0480	-0.9520	3.4000
1.2601	7.4000	8.8221	1.4221	4.6000

Error Summary

No Errors

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.1434	1.1434	0.0347	1.0944	1.2338
S	1.2597	1.2597	0.2465	0.8638	1.8542
Y0	24.40	24.40	1.04	21.90	26.07

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	14.590	12.427	17.130
20.0	7.827	6.053	10.121
10.0	5.719	3.969	8.239
5.0	4.580	2.903	7.227
0.0	2.681	1.305	5.506

08/01/2017 11:55

MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	52	1765.	33.94		
Regression	2	1174.	587.20	49.7	0.0000
Error	50	591.	11.81		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
-0.1549	30.0000	23.9847	-6.0153	1.
-0.1549	28.0000	23.9847	-4.0153	1.
-0.1549	25.0000	23.9847	-1.0153	1.
-0.1549	25.0000	23.9847	-1.0153	1.
-0.1549	25.0000	23.9847	-1.0153	1.
-0.1549	24.0000	23.9847	-0.0153	1.
-0.1549	23.0000	23.9847	0.9847	1.
-0.1549	23.0000	23.9847	0.9847	1.
-0.1549	22.0000	23.9847	1.9847	1.
-0.1549	19.0000	23.9847	4.9847	1.
0.6721	25.0000	22.6674	-2.3326	1.
0.6721	24.0000	22.6674	-1.3326	1.
0.6721	23.0000	22.6674	-0.3326	1.
0.6721	23.0000	22.6674	-0.3326	1.
0.6721	22.0000	22.6674	0.6674	1.
0.6721	22.0000	22.6674	0.6674	1.
0.6721	21.0000	22.6674	1.6674	1.
0.6721	21.0000	22.6674	1.6674	1.
0.6721	21.0000	22.6674	1.6674	1.
0.6721	21.0000	22.6674	1.6674	1.
0.8062	26.0000	20.8207	-5.1793	1.
0.8062	25.0000	20.8207	-4.1793	1.
0.8062	22.0000	20.8207	-1.1793	1.
0.8062	21.0000	20.8207	-0.1793	1.
0.8062	21.0000	20.8207	-0.1793	1.
0.8062	19.0000	20.8207	1.8207	1.

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Data Summary

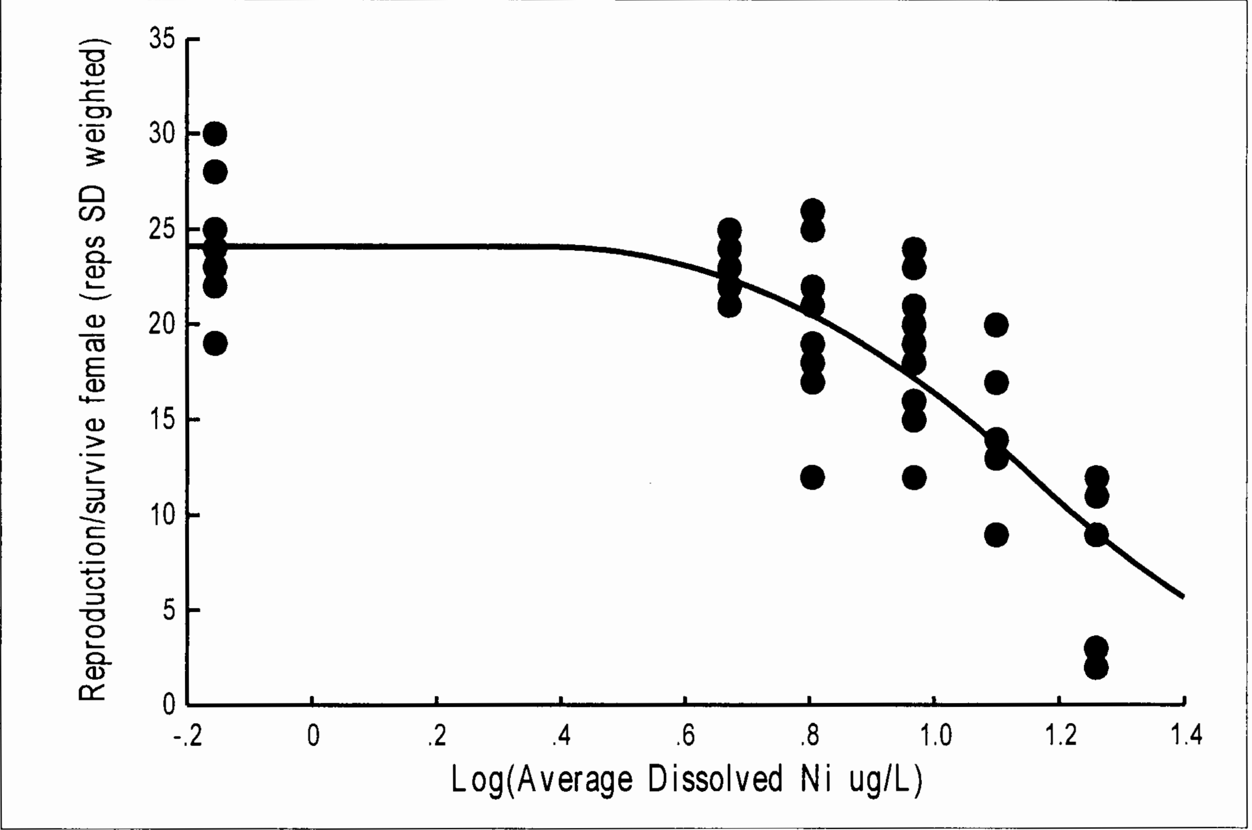
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8062	18.0000	20.8207	2.8207	1.
0.8062	18.0000	20.8207	2.8207	1.
0.8062	17.0000	20.8207	3.8207	1.
0.8062	12.0000	20.8207	8.8207	1.
0.9685	24.0000	17.5199	-6.4801	1.
0.9685	23.0000	17.5199	-5.4801	1.
0.9685	21.0000	17.5199	-3.4801	1.
0.9685	20.0000	17.5199	-2.4801	1.
0.9685	19.0000	17.5199	-1.4801	1.
0.9685	18.0000	17.5199	-0.4801	1.
0.9685	16.0000	17.5199	1.5199	1.
0.9685	15.0000	17.5199	2.5199	1.
0.9685	15.0000	17.5199	2.5199	1.
0.9685	12.0000	17.5199	5.5199	1.
1.1004	20.0000	13.9783	-6.0217	1.
1.1004	17.0000	13.9783	-3.0217	1.
1.1004	17.0000	13.9783	-3.0217	1.
1.1004	17.0000	13.9783	-3.0217	1.
1.1004	14.0000	13.9783	-0.0217	1.
1.1004	13.0000	13.9783	0.9783	1.
1.1004	13.0000	13.9783	0.9783	1.
1.1004	9.0000	13.9783	4.9783	1.
1.2601	12.0000	9.0669	-2.9331	1.
1.2601	11.0000	9.0669	-1.9331	1.
1.2601	9.0000	9.0669	0.0669	1.
1.2601	3.0000	9.0669	6.0669	1.
1.2601	2.0000	9.0669	7.0669	1.

Error Summary

No Errors

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.1434	1.1434	0.0405	1.0767	1.2395
S	1.2597	1.2597	0.2022	0.8806	1.6931
Y0	24.40	24.40	0.91	22.28	25.93

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	14.391	11.931	17.359
20.0	7.456	5.863	9.482
10.0	5.352	3.872	7.399
5.0	4.234	2.847	6.296
0.0	2.404	1.303	4.436

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	52	12096.5	232.626		
Regression	2	12050.0	6024.997	6473.	0.0000
Error	50	46.5	0.931		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
-0.1549	30.0000	24.1026	-5.8974	3.1000
-0.1549	28.0000	24.1026	-3.8974	3.1000
-0.1549	25.0000	24.1026	-0.8974	3.1000
-0.1549	25.0000	24.1026	-0.8974	3.1000
-0.1549	25.0000	24.1026	-0.8974	3.1000
-0.1549	24.0000	24.1026	0.1026	3.1000
-0.1549	23.0000	24.1026	1.1026	3.1000
-0.1549	23.0000	24.1026	1.1026	3.1000
-0.1549	22.0000	24.1026	2.1026	3.1000
-0.1549	19.0000	24.1026	5.1026	3.1000
0.6721	25.0000	22.4117	-2.5883	1.4000
0.6721	24.0000	22.4117	-1.5883	1.4000
0.6721	23.0000	22.4117	-0.5883	1.4000
0.6721	23.0000	22.4117	-0.5883	1.4000
0.6721	22.0000	22.4117	0.4117	1.4000
0.6721	22.0000	22.4117	0.4117	1.4000
0.6721	21.0000	22.4117	1.4117	1.4000
0.6721	21.0000	22.4117	1.4117	1.4000
0.6721	21.0000	22.4117	1.4117	1.4000
0.6721	21.0000	22.4117	1.4117	1.4000
0.8062	26.0000	20.4952	-5.5048	4.1000
0.8062	25.0000	20.4952	-4.5048	4.1000
0.8062	22.0000	20.4952	-1.5048	4.1000
0.8062	21.0000	20.4952	-0.5048	4.1000
0.8062	21.0000	20.4952	-0.5048	4.1000
0.8062	19.0000	20.4952	1.4952	4.1000

ASC 8/1/17

WER testing - Simulated Effluent - Ni Sim 1008 CDC (Re-analysis)

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8062	18.0000	20.4952	2.4952	4.1000
0.8062	18.0000	20.4952	2.4952	4.1000
0.8062	17.0000	20.4952	3.4952	4.1000
0.8062	12.0000	20.4952	8.4952	4.1000
0.9685	24.0000	17.2152	-6.7848	3.8000
0.9685	23.0000	17.2152	-5.7848	3.8000
0.9685	21.0000	17.2152	-3.7848	3.8000
0.9685	20.0000	17.2152	-2.7848	3.8000
0.9685	19.0000	17.2152	-1.7848	3.8000
0.9685	18.0000	17.2152	-0.7848	3.8000
0.9685	16.0000	17.2152	1.2152	3.8000
0.9685	15.0000	17.2152	2.2152	3.8000
0.9685	15.0000	17.2152	2.2152	3.8000
0.9685	12.0000	17.2152	5.2152	3.8000
1.1004	20.0000	13.7755	-6.2245	5.5000
1.1004	17.0000	13.7755	-3.2245	5.5000
1.1004	17.0000	13.7755	-3.2245	5.5000
1.1004	17.0000	13.7755	-3.2245	5.5000
1.1004	14.0000	13.7755	-0.2245	5.5000
1.1004	13.0000	13.7755	0.7755	5.5000
1.1004	13.0000	13.7755	0.7755	5.5000
1.1004	9.0000	13.7755	4.7755	5.5000
1.2601	12.0000	9.0961	-2.9039	4.0000
1.2601	11.0000	9.0961	-1.9039	4.0000
1.2601	9.0000	9.0961	0.0961	4.0000
1.2601	3.0000	9.0961	6.0961	4.0000
1.2601	2.0000	9.0961	7.0961	4.0000

Error Summary

No Errors

ASC 8/1/17

Project: Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*

Sponsor: Sanitary District of Decatur

Oregon State University Aquatic Toxicology Lab

Supplemental: Statistical Re-Analysis Exercise

Nickel WER without DOC

Test ID: Ni WER 1132R CDC

					Per original female		Per surviving female	
Nominal Treatment µg/L Ni	**Measured (Average) Dissolved Ni µg/L**	**Rep**	**Survival**	**total # of neonates**	**Average**	**Std Dev**	**Average**	**Std Dev**
0	1.3	A	1	20	26.9	6.8	26.9	6.8
0	1.3	B	1	28				
0	1.3	C	1	19				
0	1.3	D	1	29				
0	1.3	E	1	26				
0	1.3	F	1	31				
0	1.3	G	1	21				
0	1.3	H	1	35				
0	1.3	I	1	21				
0	1.3	J	1	39				
2.1	2.9	A	1	32	28.1	5.5	28.1	5.5
2.1	2.9	B	1	31				
2.1	2.9	C	1	27				
2.1	2.9	D	1	32				
2.1	2.9	E	1	23				
2.1	2.9	F	1	32				
2.1	2.9	G	1	31				
2.1	2.9	H	1	34				
2.1	2.9	I	1	21				
2.1	2.9	J	1	18				
2.9	3.5	A	1	36	29.2	5.5	29.2	5.5
2.9	3.5	B	1	32				
2.9	3.5	C	1	19				
2.9	3.5	D	1	30				
2.9	3.5	E	1	20				
2.9	3.5	F	1	30				
2.9	3.5	G	1	28				
2.9	3.5	H	1	32				
2.9	3.5	I	1	33				
2.9	3.5	J	1	32				
4.2	4.5	A	1	25	27.1	9.0	29.8	3.3
4.2	4.5	B	1	27				
4.2	4.5	C	1	35				
4.2	4.5	D	1	31				
4.2	4.5	E	1	30				
4.2	4.5	F	1	33				
4.2	4.5	G	**0**	**3**				
4.2	4.5	H	1	27				
4.2	4.5	I	1	28				
4.2	4.5	J	1	32				

ASC 8/1/17

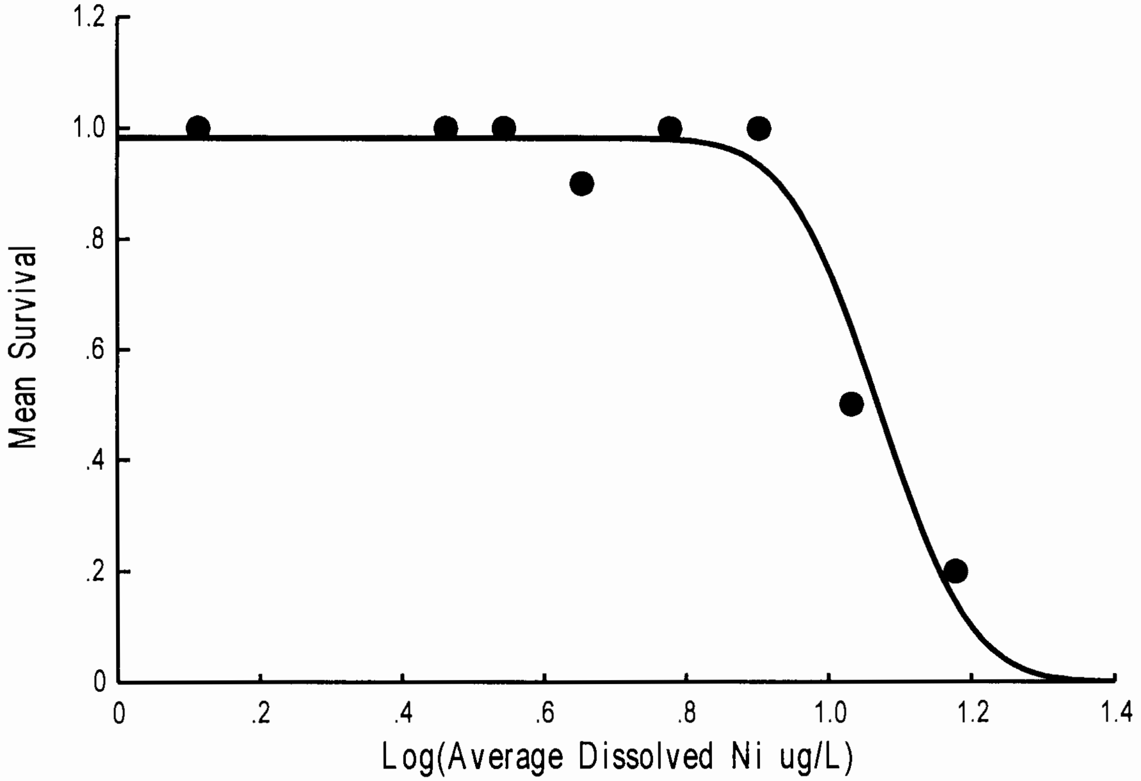
Project: Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*
Sponsor: Sanitary District of Decatur
Oregon State University Aquatic Toxicology Lab
Supplemental: Statistical Re-Analysis Exercise

Nickel WER without DOC
Test ID: Ni WER 1132R CDC

					Per original female		Per surviving female	
Nominal Treatment µg/L Ni	**Measured (Average) Dissolved Ni µg/L**	**Rep**	**Survival**	**total # of neonates**	**Average**	**Std Dev**	**Average**	**Std Dev**
6.0	6.0	A	1	28	28.6	2.2	28.6	2.2
6.0	6.0	B	1	32				
6.0	6.0	C	1	30				
6.0	6.0	D	1	31				
6.0	6.0	E	1	25				
6.0	6.0	F	1	27				
6.0	6.0	G	1	26				
6.0	6.0	H	1	30				
6.0	6.0	I	1	28				
6.0	6.0	J	1	29				
8.5	8.0	A	1	32	17.2	10.9	17.2	10.9
8.5	8.0	B	1	24				
8.5	8.0	C	1	0				
8.5	8.0	D	1	6				
8.5	8.0	E	1	18				
8.5	8.0	F	1	7				
8.5	8.0	G	1	25				
8.5	8.0	H	1	12				
8.5	8.0	I	1	31				
8.5	8.0	J	1	17				
12.2	10.8	A	1	5	12.0	6.5	13.0	7.8
12.2	10.8	B	1	11				
12.2	10.8	C	**0**	**5**				
12.2	10.8	D	1	13				
12.2	10.8	E	**0**	**6**				
12.2	10.8	F	**0**	**16**				
12.2	10.8	G	1	10				
12.2	10.8	H	**0**	**17**				
12.2	10.8	I	1	26				
12.2	10.8	J	**0**	**11**				
17.4	15.1	A	**0**	**3**	9.2	6.0	13.5	2.1
17.4	15.1	B	**0**	**9**				
17.4	15.1	C	**0**	**0**				
17.4	15.1	D	1	15				
17.4	15.1	E	**0**	**4**				
17.4	15.1	F	**0**	**5**				
17.4	15.1	G	**0**	**11**				
17.4	15.1	H	**0**	**18**				
17.4	15.1	I	**0**	**15**				
17.4	15.1	J	1	12				

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent no DOC added: Ni WER 1132R



Parameter Summary (Gaussian Tolerance Distribution Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.0000	1.0000	0.0304	1.0051	1.1387
StdDev	0.10000	0.10000	0.03288	0.07093	0.17816
Y0	1.0000	1.0000	0.0189	0.8952	0.9997

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	11.800	10.117	13.763
20.0	9.693	7.805	12.036
10.0	8.745	6.526	11.719
5.0	8.033	5.500	11.733

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent no DOC added: Ni WER 1132R

Data Summary

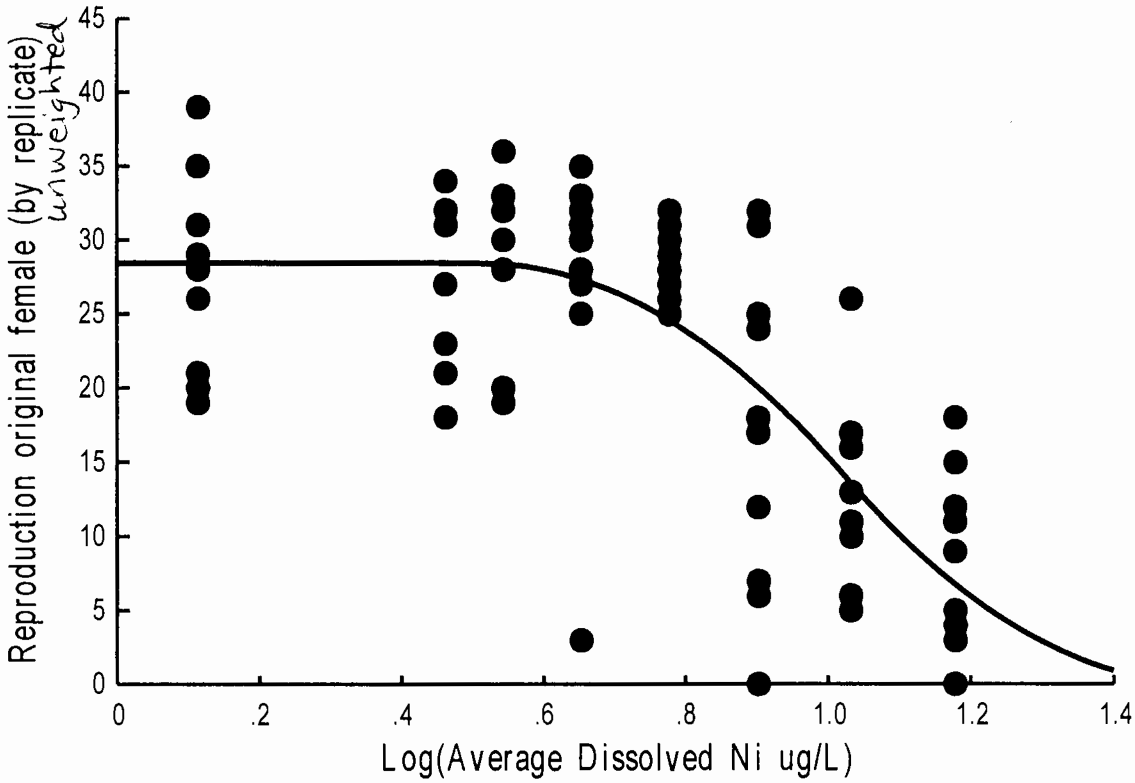
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Total N
0.1139	1.0000	0.9824	-0.0176	10.
0.4624	1.0000	0.9824	-0.0176	10.
0.5441	1.0000	0.9824	-0.0176	10.
0.6532	0.9000	0.9824	0.0824	10.
0.7782	1.0000	0.9806	-0.0194	10.
0.9031	1.0000	0.9351	-0.0649	10.
1.0334	0.5000	0.6363	0.1363	10.
1.1790	0.2000	0.1433	-0.0567	10.

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.0515	1.0515	0.0310	0.9600	1.0837
S	2.689	2.689	0.4285	1.1159	2.8223
Y0	27.98	27.98	1.31	25.82	31.03

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	10.516	9.121	12.125
20.0	6.842	5.355	8.743
10.0	5.510	3.923	7.737
5.0	4.727	3.089	7.235
0.0	3.266	1.474	7.234

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	79	8202.	103.8		
Regression	2	4329.	2164.3	43.0	0.0000
Error	77	3874.	50.3		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	39.0000	28.4237	-10.5763	1.
0.1139	35.0000	28.4237	-6.5763	1.
0.1139	31.0000	28.4237	-2.5763	1.
0.1139	29.0000	28.4237	-0.5763	1.
0.1139	28.0000	28.4237	0.4237	1.
0.1139	26.0000	28.4237	2.4237	1.
0.1139	21.0000	28.4237	7.4237	1.
0.1139	21.0000	28.4237	7.4237	1.
0.1139	20.0000	28.4237	8.4237	1.
0.1139	19.0000	28.4237	9.4237	1.
0.4624	34.0000	28.4237	-5.5763	1.
0.4624	32.0000	28.4237	-3.5763	1.
0.4624	32.0000	28.4237	-3.5763	1.
0.4624	32.0000	28.4237	-3.5763	1.
0.4624	31.0000	28.4237	-2.5763	1.
0.4624	31.0000	28.4237	-2.5763	1.
0.4624	27.0000	28.4237	1.4237	1.
0.4624	23.0000	28.4237	5.4237	1.
0.4624	21.0000	28.4237	7.4237	1.
0.4624	18.0000	28.4237	10.4237	1.
0.5441	36.0000	28.3739	-7.6261	1.
0.5441	33.0000	28.3739	-4.6261	1.
0.5441	32.0000	28.3739	-3.6261	1.
0.5441	32.0000	28.3739	-3.6261	1.
0.5441	32.0000	28.3739	-3.6261	1.
0.5441	30.0000	28.3739	-1.6261	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.5441	30.0000	28.3739	-1.6261	1.
0.5441	28.0000	28.3739	0.3739	1.
0.5441	20.0000	28.3739	8.3739	1.
0.5441	19.0000	28.3739	9.3739	1.
0.6532	35.0000	27.3558	-7.6442	1.
0.6532	33.0000	27.3558	-5.6442	1.
0.6532	32.0000	27.3558	-4.6442	1.
0.6532	31.0000	27.3558	-3.6442	1.
0.6532	30.0000	27.3558	-2.6442	1.
0.6532	28.0000	27.3558	-0.6442	1.
0.6532	27.0000	27.3558	0.3558	1.
0.6532	27.0000	27.3558	0.3558	1.
0.6532	25.0000	27.3558	2.3558	1.
0.6532	3.0000	27.3558	24.3558	1.
0.7782	32.0000	24.5788	-7.4212	1.
0.7782	31.0000	24.5788	-6.4212	1.
0.7782	30.0000	24.5788	-5.4212	1.
0.7782	30.0000	24.5788	-5.4212	1.
0.7782	29.0000	24.5788	-4.4212	1.
0.7782	28.0000	24.5788	-3.4212	1.
0.7782	28.0000	24.5788	-3.4212	1.
0.7782	27.0000	24.5788	-2.4212	1.
0.7782	26.0000	24.5788	-1.4212	1.
0.7782	25.0000	24.5788	-0.4212	1.
0.9031	32.0000	20.0814	-11.9186	1.
0.9031	31.0000	20.0814	-10.9186	1.
0.9031	25.0000	20.0814	-4.9186	1.
0.9031	24.0000	20.0814	-3.9186	1.
0.9031	18.0000	20.0814	2.0814	1.
0.9031	17.0000	20.0814	3.0814	1.
0.9031	12.0000	20.0814	8.0814	1.
0.9031	7.0000	20.0814	13.0814	1.
0.9031	6.0000	20.0814	14.0814	1.
0.9031	0.0000	20.0814	20.0814	1.
1.0334	26.0000	13.5713	-12.4287	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

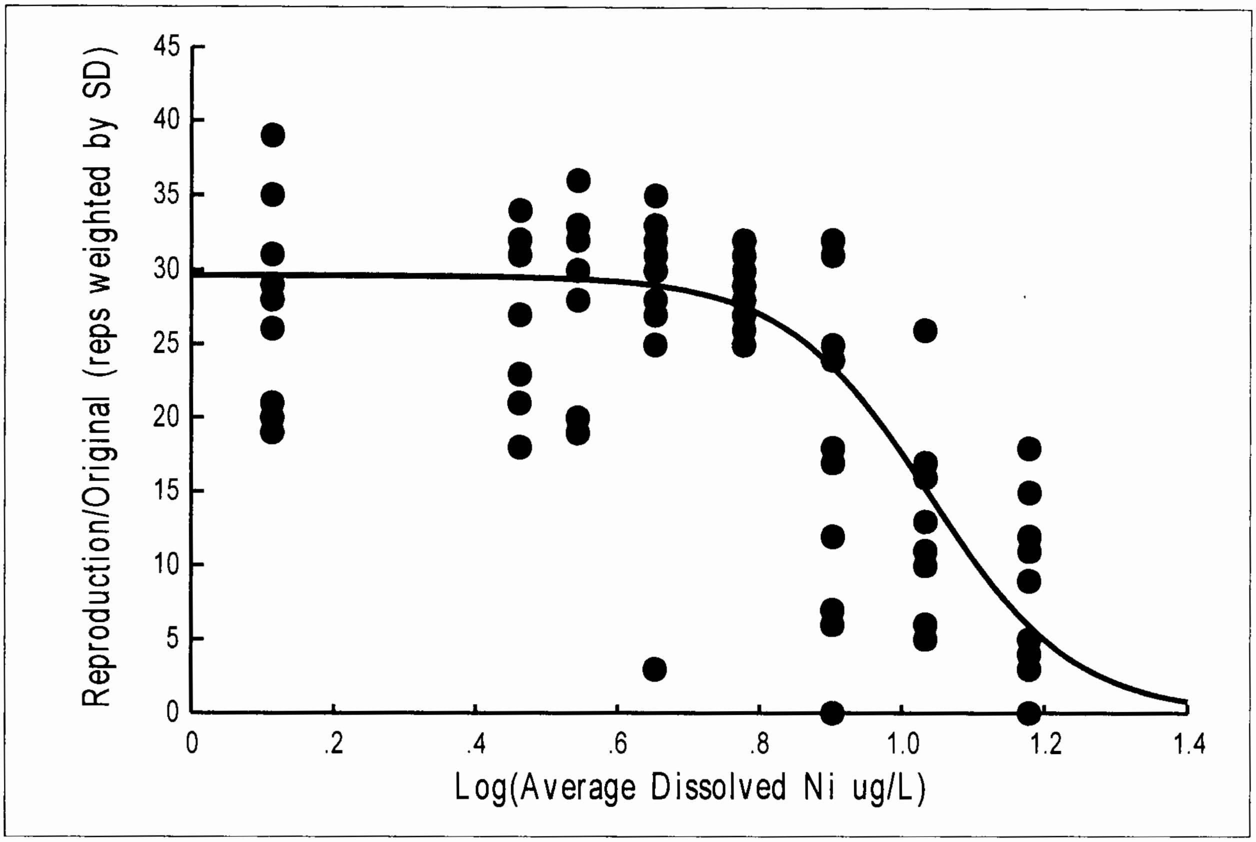
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.0334	17.0000	13.5713	-3.4287	1.
1.0334	16.0000	13.5713	-2.4287	1.
1.0334	13.0000	13.5713	0.5713	1.
1.0334	11.0000	13.5713	2.5713	1.
1.0334	11.0000	13.5713	2.5713	1.
1.0334	10.0000	13.5713	3.5713	1.
1.0334	6.0000	13.5713	7.5713	1.
1.0334	5.0000	13.5713	8.5713	1.
1.0334	5.0000	13.5713	8.5713	1.
1.1790	18.0000	6.7779	-11.2221	1.
1.1790	15.0000	6.7779	-8.2221	1.
1.1790	15.0000	6.7779	-8.2221	1.
1.1790	12.0000	6.7779	-5.2221	1.
1.1790	11.0000	6.7779	-4.2221	1.
1.1790	9.0000	6.7779	-2.2221	1.
1.1790	5.0000	6.7779	1.7779	1.
1.1790	4.0000	6.7779	2.7779	1.
1.1790	3.0000	6.7779	3.7779	1.
1.1790	0.0000	6.7779	6.7779	1.

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R



Parameter Summary (Logistic Equation Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.0515	1.0515	0.0249	0.9894	1.0887
S	2.689	2.689	0.488	1.508	3.453
Y0	27.98	27.98	1.11	27.42	31.84

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	10.942	9.759	12.267
20.0	7.932	6.586	9.551
10.0	6.571	5.124	8.426
5.0	5.525	4.049	7.539

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	79	37153.2	470.293		
Regression	2	37067.0	18533.486	16558.	0.0000
Error	77	86.2	1.119		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	39.0000	29.6281	-9.3719	6.8000
0.1139	35.0000	29.6281	-5.3719	6.8000
0.1139	31.0000	29.6281	-1.3719	6.8000
0.1139	29.0000	29.6281	0.6281	6.8000
0.1139	28.0000	29.6281	1.6281	6.8000
0.1139	26.0000	29.6281	3.6281	6.8000
0.1139	21.0000	29.6281	8.6281	6.8000
0.1139	21.0000	29.6281	8.6281	6.8000
0.1139	20.0000	29.6281	9.6281	6.8000
0.1139	19.0000	29.6281	10.6281	6.8000
0.4624	34.0000	29.5344	-4.4656	5.5000
0.4624	32.0000	29.5344	-2.4656	5.5000
0.4624	32.0000	29.5344	-2.4656	5.5000
0.4624	32.0000	29.5344	-2.4656	5.5000
0.4624	31.0000	29.5344	-1.4656	5.5000
0.4624	31.0000	29.5344	-1.4656	5.5000
0.4624	27.0000	29.5344	2.5344	5.5000
0.4624	23.0000	29.5344	6.5344	5.5000
0.4624	21.0000	29.5344	8.5344	5.5000
0.4624	18.0000	29.5344	11.5344	5.5000
0.5441	36.0000	29.4145	-6.5855	5.5000
0.5441	33.0000	29.4145	-3.5855	5.5000
0.5441	32.0000	29.4145	-2.5855	5.5000
0.5441	32.0000	29.4145	-2.5855	5.5000
0.5441	32.0000	29.4145	-2.5855	5.5000
0.5441	30.0000	29.4145	-0.5855	5.5000

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.5441	30.0000	29.4145	-0.5855	5.5000
0.5441	28.0000	29.4145	1.4145	5.5000
0.5441	20.0000	29.4145	9.4145	5.5000
0.5441	19.0000	29.4145	10.4145	5.5000
0.6532	35.0000	29.0005	-5.9995	9.0000
0.6532	33.0000	29.0005	-3.9995	9.0000
0.6532	32.0000	29.0005	-2.9995	9.0000
0.6532	31.0000	29.0005	-1.9995	9.0000
0.6532	30.0000	29.0005	-0.9995	9.0000
0.6532	28.0000	29.0005	1.0005	9.0000
0.6532	27.0000	29.0005	2.0005	9.0000
0.6532	27.0000	29.0005	2.0005	9.0000
0.6532	25.0000	29.0005	4.0005	9.0000
0.6532	3.0000	29.0005	26.0005	9.0000
0.7782	32.0000	27.5611	-4.4389	2.2000
0.7782	31.0000	27.5611	-3.4389	2.2000
0.7782	30.0000	27.5611	-2.4389	2.2000
0.7782	30.0000	27.5611	-2.4389	2.2000
0.7782	29.0000	27.5611	-1.4389	2.2000
0.7782	28.0000	27.5611	-0.4389	2.2000
0.7782	28.0000	27.5611	-0.4389	2.2000
0.7782	27.0000	27.5611	0.5611	2.2000
0.7782	26.0000	27.5611	1.5611	2.2000
0.7782	25.0000	27.5611	2.5611	2.2000
0.9031	32.0000	23.5274	-8.4726	10.9000
0.9031	31.0000	23.5274	-7.4726	10.9000
0.9031	25.0000	23.5274	-1.4726	10.9000
0.9031	24.0000	23.5274	-0.4726	10.9000
0.9031	18.0000	23.5274	5.5274	10.9000
0.9031	17.0000	23.5274	6.5274	10.9000
0.9031	12.0000	23.5274	11.5274	10.9000
0.9031	7.0000	23.5274	16.5274	10.9000
0.9031	6.0000	23.5274	17.5274	10.9000
0.9031	0.0000	23.5274	23.5274	10.9000
1.0334	26.0000	15.2317	-10.7683	6.5000

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

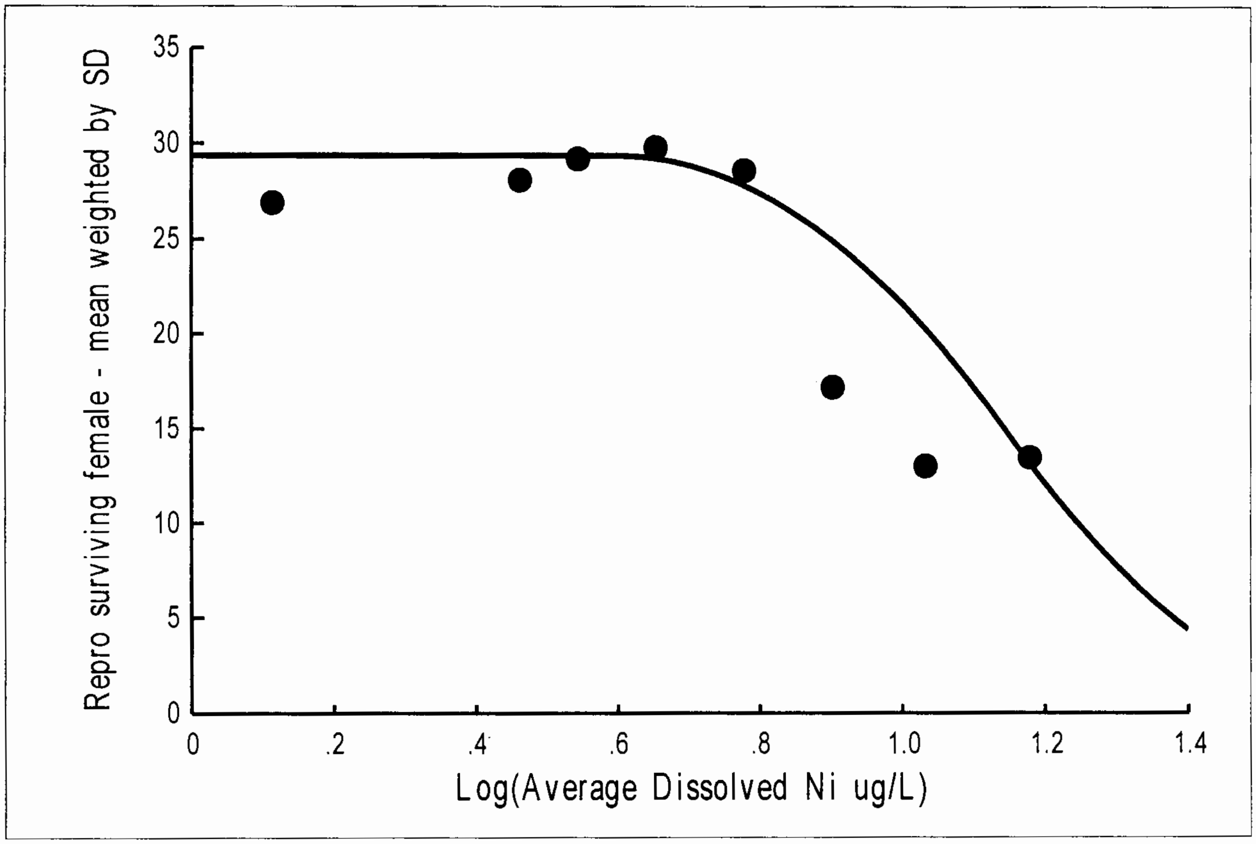
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.0334	17.0000	15.2317	-1.7683	6.5000
1.0334	16.0000	15.2317	-0.7683	6.5000
1.0334	13.0000	15.2317	2.2317	6.5000
1.0334	11.0000	15.2317	4.2317	6.5000
1.0334	11.0000	15.2317	4.2317	6.5000
1.0334	10.0000	15.2317	5.2317	6.5000
1.0334	6.0000	15.2317	9.2317	6.5000
1.0334	5.0000	15.2317	10.2317	6.5000
1.0334	5.0000	15.2317	10.2317	6.5000
1.1790	18.0000	5.9186	-12.0814	6.0000
1.1790	15.0000	5.9186	-9.0814	6.0000
1.1790	15.0000	5.9186	-9.0814	6.0000
1.1790	12.0000	5.9186	-6.0814	6.0000
1.1790	11.0000	5.9186	-5.0814	6.0000
1.1790	9.0000	5.9186	-3.0814	6.0000
1.1790	5.0000	5.9186	0.9186	6.0000
1.1790	4.0000	5.9186	1.9186	6.0000
1.1790	3.0000	5.9186	2.9186	6.0000
1.1790	0.0000	5.9186	5.9186	6.0000

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	0.9918	0.9918	0.0301	1.0723	1.2271
S	3.965	3.965	0.4986	0.5363	3.0998
Y0	28.52	28.52	1.65	25.14	33.60

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	14.115	11.810	16.868
20.0	8.861	5.755	13.644
10.0	7.008	3.883	12.648
5.0	5.937	2.920	12.071
0.0	3.978	1.403	11.276

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MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	7	2424.459	346.351		
Regression	2	2422.733	1211.366	3508.	0.0000
Error	5	1.726	0.345		

Data Summary

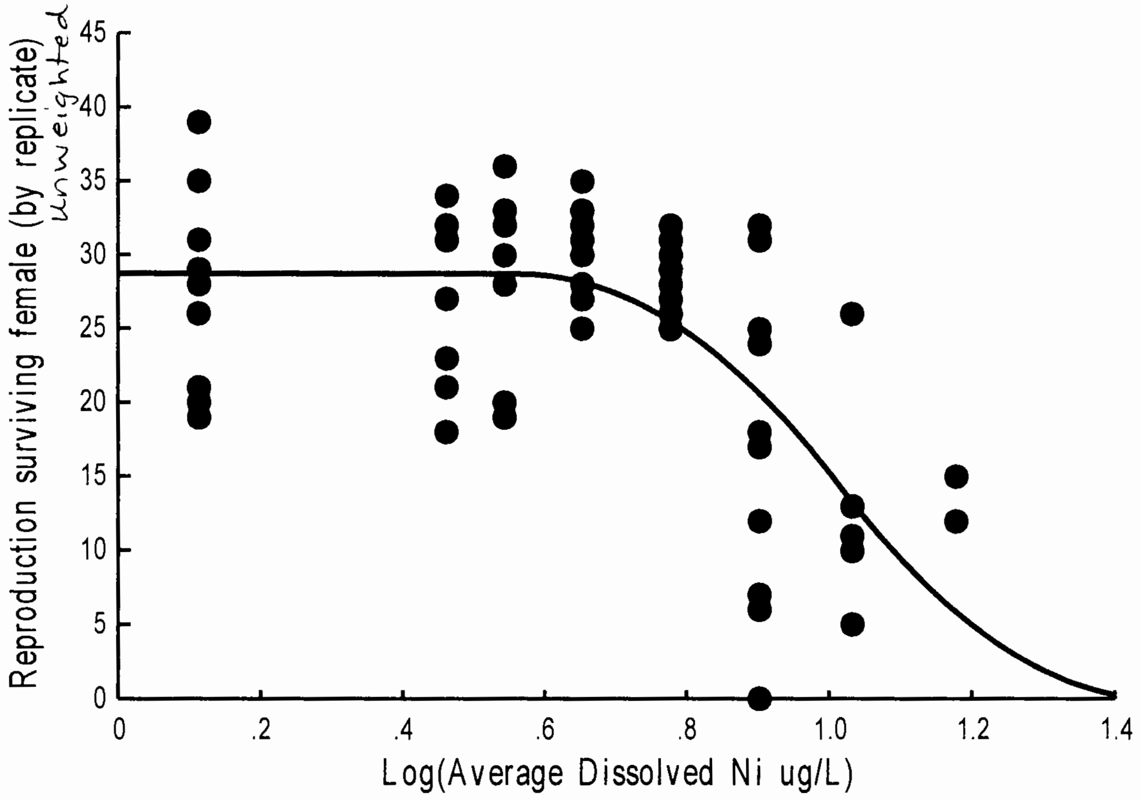
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	26.9000	29.3713	2.4713	6.8000
0.4624	28.1000	29.3713	1.2713	5.5000
0.5441	29.2000	29.3713	0.1713	5.5000
0.6532	29.8000	29.2319	-0.5681	3.3000
0.7782	28.6000	27.8243	-0.7757	2.2000
0.9031	17.2000	24.9013	7.7013	10.9000
1.0334	13.0000	20.2371	7.2371	7.8000
1.1790	13.5000	13.1624	-0.3376	2.1000

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	0.9918	0.9918	0.0359	0.9444	1.0877
S	3.964	3.964	0.597	1.026	3.413
Y0	28.52	28.52	1.17	26.38	31.05

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	10.377	8.798	12.239
20.0	7.087	5.751	8.733
10.0	5.848	4.332	7.894
5.0	5.105	3.464	7.523
0.0	3.677	1.779	7.601

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	65	4652.	71.6		
Regression	2	1841.	920.5	20.6	0.0000
Error	63	2812.	44.6		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	39.0000	28.7108	-10.2892	1.
0.1139	35.0000	28.7108	-6.2892	1.
0.1139	31.0000	28.7108	-2.2892	1.
0.1139	29.0000	28.7108	-0.2892	1.
0.1139	28.0000	28.7108	0.7108	1.
0.1139	26.0000	28.7108	2.7108	1.
0.1139	21.0000	28.7108	7.7108	1.
0.1139	21.0000	28.7108	7.7108	1.
0.1139	20.0000	28.7108	8.7108	1.
0.1139	19.0000	28.7108	9.7108	1.
0.4624	34.0000	28.7108	-5.2892	1.
0.4624	32.0000	28.7108	-3.2892	1.
0.4624	32.0000	28.7108	-3.2892	1.
0.4624	32.0000	28.7108	-3.2892	1.
0.4624	31.0000	28.7108	-2.2892	1.
0.4624	31.0000	28.7108	-2.2892	1.
0.4624	27.0000	28.7108	1.7108	1.
0.4624	23.0000	28.7108	5.7108	1.
0.4624	21.0000	28.7108	7.7108	1.
0.4624	18.0000	28.7108	10.7108	1.
0.5441	36.0000	28.7108	-7.2892	1.
0.5441	33.0000	28.7108	-4.2892	1.
0.5441	32.0000	28.7108	-3.2892	1.
0.5441	32.0000	28.7108	-3.2892	1.
0.5441	32.0000	28.7108	-3.2892	1.
0.5441	30.0000	28.7108	-1.2892	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.5441	30.0000	28.7108	-1.2892	1.
0.5441	28.0000	28.7108	0.7108	1.
0.5441	20.0000	28.7108	8.7108	1.
0.5441	19.0000	28.7108	9.7108	1.
0.6532	35.0000	28.1668	-6.8332	1.
0.6532	33.0000	28.1668	-4.8332	1.
0.6532	32.0000	28.1668	-3.8332	1.
0.6532	31.0000	28.1668	-2.8332	1.
0.6532	30.0000	28.1668	-1.8332	1.
0.6532	28.0000	28.1668	0.1668	1.
0.6532	27.0000	28.1668	1.1668	1.
0.6532	27.0000	28.1668	1.1668	1.
0.6532	25.0000	28.1668	3.1668	1.
0.7782	32.0000	25.5131	-6.4869	1.
0.7782	31.0000	25.5131	-5.4869	1.
0.7782	30.0000	25.5131	-4.4869	1.
0.7782	30.0000	25.5131	-4.4869	1.
0.7782	29.0000	25.5131	-3.4869	1.
0.7782	28.0000	25.5131	-2.4869	1.
0.7782	28.0000	25.5131	-2.4869	1.
0.7782	27.0000	25.5131	-1.4869	1.
0.7782	26.0000	25.5131	-0.4869	1.
0.7782	25.0000	25.5131	0.5131	1.
0.9031	32.0000	20.6515	-11.3485	1.
0.9031	31.0000	20.6515	-10.3485	1.
0.9031	25.0000	20.6515	-4.3485	1.
0.9031	24.0000	20.6515	-3.3485	1.
0.9031	18.0000	20.6515	2.6515	1.
0.9031	17.0000	20.6515	3.6515	1.
0.9031	12.0000	20.6515	8.6515	1.
0.9031	7.0000	20.6515	13.6515	1.
0.9031	6.0000	20.6515	14.6515	1.
0.9031	0.0000	20.6515	20.6515	1.
1.0334	26.0000	13.2698	-12.7302	1.
1.0334	13.0000	13.2698	0.2698	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

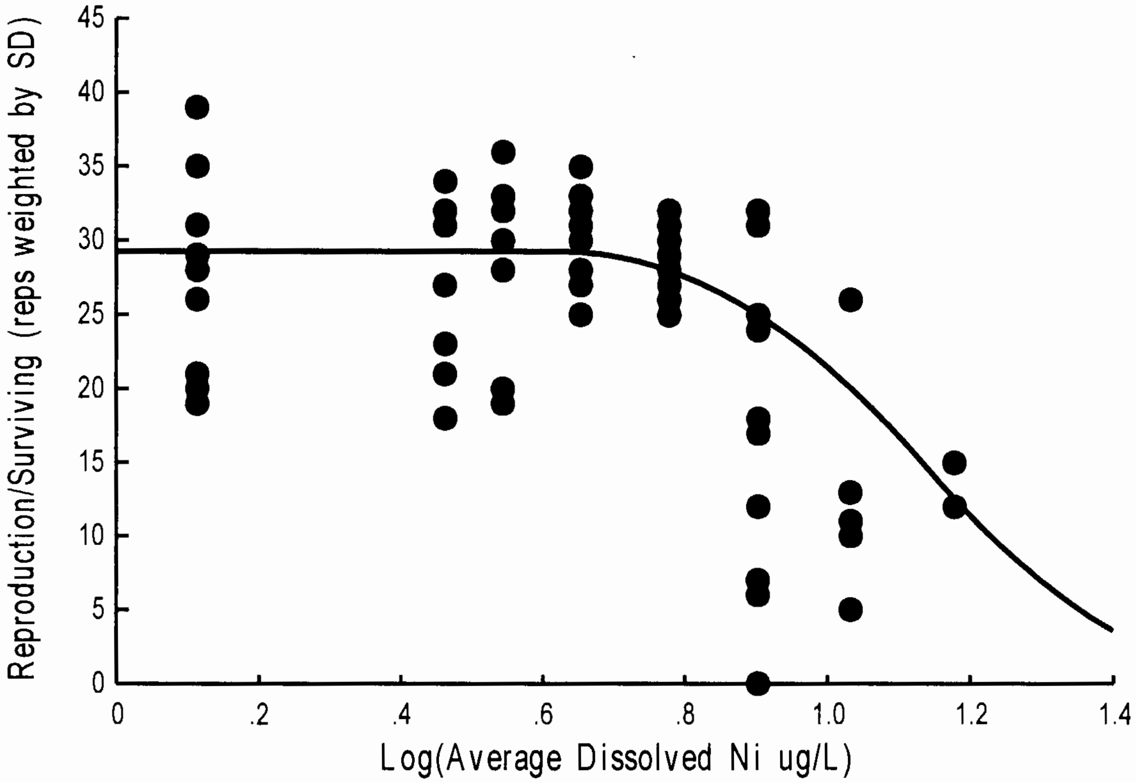
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.0334	11.0000	13.2698	2.2698	1.
1.0334	10.0000	13.2698	3.2698	1.
1.0334	5.0000	13.2698	8.2698	1.
1.1790	15.0000	5.8503	-9.1497	1.
1.1790	12.0000	5.8503	-6.1497	1.

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	0.9918	0.9918	0.0283	1.0838	1.1970
S	3.964	3.964	0.3382	1.2677	2.6196
Y0	28.52	28.52	0.89	27.48	31.04

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	13.817	12.129	15.738
20.0	8.939	7.384	10.822
10.0	7.178	5.580	9.234
5.0	6.146	4.547	8.308
0.0	4.226	2.661	6.711

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MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	65	29355.3	451.620		
Regression	2	29284.6	14642.285	13041.	0.0000
Error	63	70.7	1.123		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	39.0000	29.2610	-9.7390	6.8000
0.1139	35.0000	29.2610	-5.7390	6.8000
0.1139	31.0000	29.2610	-1.7390	6.8000
0.1139	29.0000	29.2610	0.2610	6.8000
0.1139	28.0000	29.2610	1.2610	6.8000
0.1139	26.0000	29.2610	3.2610	6.8000
0.1139	21.0000	29.2610	8.2610	6.8000
0.1139	21.0000	29.2610	8.2610	6.8000
0.1139	20.0000	29.2610	9.2610	6.8000
0.1139	19.0000	29.2610	10.2610	6.8000
0.4624	34.0000	29.2610	-4.7390	5.5000
0.4624	32.0000	29.2610	-2.7390	5.5000
0.4624	32.0000	29.2610	-2.7390	5.5000
0.4624	32.0000	29.2610	-2.7390	5.5000
0.4624	31.0000	29.2610	-1.7390	5.5000
0.4624	31.0000	29.2610	-1.7390	5.5000
0.4624	27.0000	29.2610	2.2610	5.5000
0.4624	23.0000	29.2610	6.2610	5.5000
0.4624	21.0000	29.2610	8.2610	5.5000
0.4624	18.0000	29.2610	11.2610	5.5000
0.5441	36.0000	29.2610	-6.7390	5.5000
0.5441	33.0000	29.2610	-3.7390	5.5000
0.5441	32.0000	29.2610	-2.7390	5.5000
0.5441	32.0000	29.2610	-2.7390	5.5000
0.5441	32.0000	29.2610	-2.7390	5.5000
0.5441	30.0000	29.2610	-0.7390	5.5000

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.5441	30.0000	29.2610	-0.7390	5.5000
0.5441	28.0000	29.2610	1.2610	5.5000
0.5441	20.0000	29.2610	9.2610	5.5000
0.5441	19.0000	29.2610	10.2610	5.5000
0.6532	35.0000	29.2197	-5.7803	3.3000
0.6532	33.0000	29.2197	-3.7803	3.3000
0.6532	32.0000	29.2197	-2.7803	3.3000
0.6532	31.0000	29.2197	-1.7803	3.3000
0.6532	30.0000	29.2197	-0.7803	3.3000
0.6532	28.0000	29.2197	1.2197	3.3000
0.6532	27.0000	29.2197	2.2197	3.3000
0.6532	27.0000	29.2197	2.2197	3.3000
0.6532	25.0000	29.2197	4.2197	3.3000
0.7782	32.0000	27.9798	-4.0202	2.2000
0.7782	31.0000	27.9798	-3.0202	2.2000
0.7782	30.0000	27.9798	-2.0202	2.2000
0.7782	30.0000	27.9798	-2.0202	2.2000
0.7782	29.0000	27.9798	-1.0202	2.2000
0.7782	28.0000	27.9798	-0.0202	2.2000
0.7782	28.0000	27.9798	-0.0202	2.2000
0.7782	27.0000	27.9798	0.9798	2.2000
0.7782	26.0000	27.9798	1.9798	2.2000
0.7782	25.0000	27.9798	2.9798	2.2000
0.9031	32.0000	25.0143	-6.9857	10.9000
0.9031	31.0000	25.0143	-5.9857	10.9000
0.9031	25.0000	25.0143	0.0143	10.9000
0.9031	24.0000	25.0143	1.0143	10.9000
0.9031	18.0000	25.0143	7.0143	10.9000
0.9031	17.0000	25.0143	8.0143	10.9000
0.9031	12.0000	25.0143	13.0143	10.9000
0.9031	7.0000	25.0143	18.0143	10.9000
0.9031	6.0000	25.0143	19.0143	10.9000
0.9031	0.0000	25.0143	25.0143	10.9000
1.0334	26.0000	20.0820	-5.9180	7.8000
1.0334	13.0000	20.0820	7.0820	7.8000

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.0334	11.0000	20.0820	9.0820	7.8000
1.0334	10.0000	20.0820	10.0820	7.8000
1.0334	5.0000	20.0820	15.0820	7.8000
1.1790	15.0000	12.5187	-2.4813	2.1000
1.1790	12.0000	12.5187	0.5187	2.1000

Error Summary

No Errors

ASC 8/1/17

Project: Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*
Sponsor: Sanitary District of Decatur
Oregon State University Aquatic Toxicology Lab
Supplemental: Statistical Re-Analysis Exercise

Nickel WER with added DOC
Test ID: Ni WER 1126 CDC

					Per original female		Per surviving female	
Nominal Treatment µg/L Ni	**Measured (Average) Dissolved Ni µg/L**	**Rep**	**Survival**	**total # of neonates**	**Average**	**Std Dev**	**Average**	**Std Dev**
0	1.6	A	1	33	39.4	4.9	39.4	4.9
0	1.6	B	1	44				
0	1.6	C	1	37				
0	1.6	D	1	39				
0	1.6	E	1	40				
0	1.6	F	1	47				
0	1.6	G	1	31				
0	1.6	H	1	40				
0	1.6	I	1	39				
0	1.6	J	1	44				
4.5	5.4	A	1	41	37.1	13.2	41.2	2.2
4.5	5.4	B	1	44				
4.5	5.4	C	1	38				
4.5	5.4	D	**0**	**0**				
4.5	5.4	E	1	38				
4.5	5.4	F	1	42				
4.5	5.4	G	1	42				
4.5	5.4	H	1	40				
4.5	5.4	I	1	43				
4.5	5.4	J	1	43				
6.5	6.8	A	1	43	37.7	10.1	40.8	2.7
6.5	6.8	B	1	41				
6.5	6.8	C	1	45				
6.5	6.8	D	1	43				
6.5	6.8	E	**0**	**10**				
6.5	6.8	F	1	42				
6.5	6.8	G	1	38				
6.5	6.8	H	1	38				
6.5	6.8	I	1	37				
6.5	6.8	J	1	40				
9.2	9.0	A	1	39	37.5	2.3	37.5	2.3
9.2	9.0	B	1	33				
9.2	9.0	C	1	41				
9.2	9.0	D	1	35				
9.2	9.0	E	1	37				
9.2	9.0	F	1	39				
9.2	9.0	G	1	39				
9.2	9.0	H	1	36				
9.2	9.0	I	1	38				
9.2	9.0	J	1	38				

ASC 8/2/17

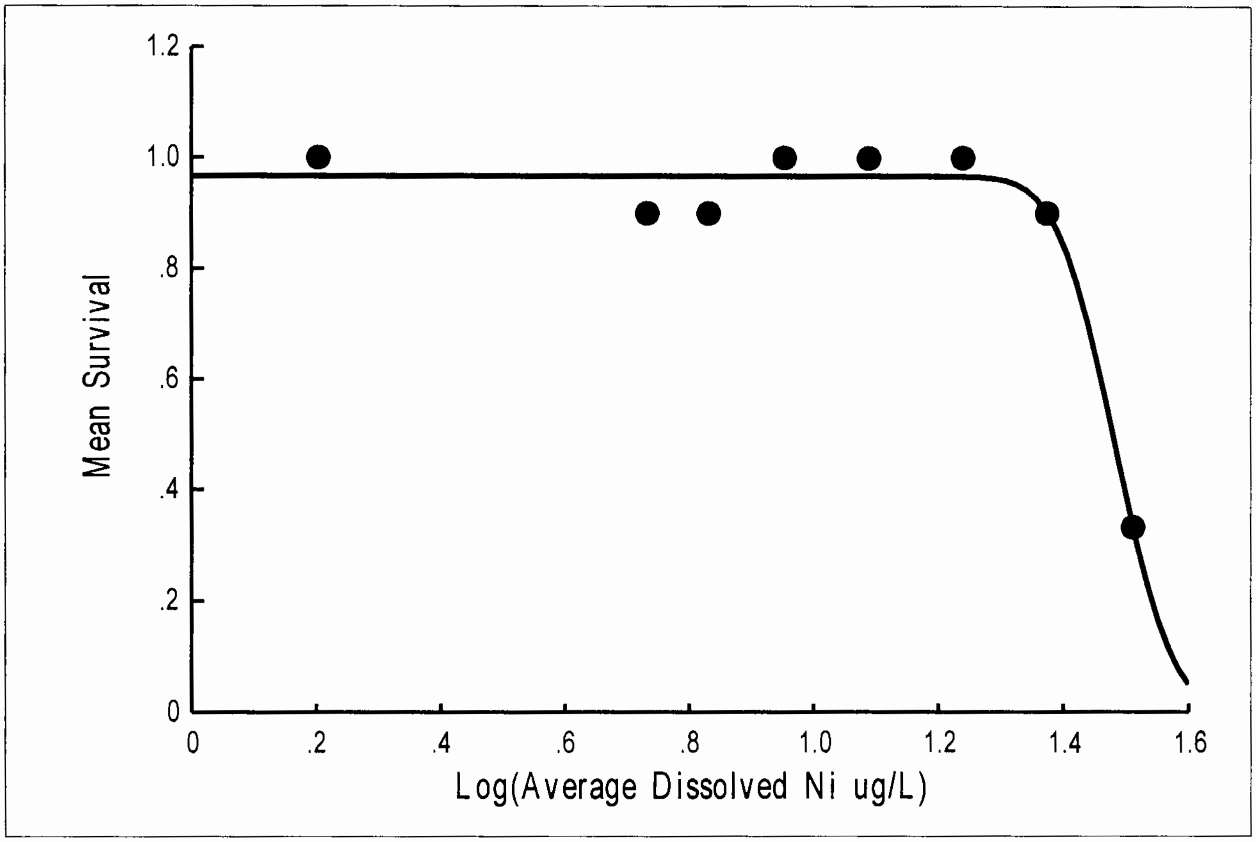
Project: Chronic toxicity of a nickel-spiked simulated effluent, with and without dissolved organic carbon (DOC), to the cladoceran, *Ceriodaphnia dubia*
Sponsor: Sanitary District of Decatur
Oregon State University Aquatic Toxicology Lab
Supplemental: Statistical Re-Analysis Exercise

Nickel WER with added DOC
Test ID: Ni WER 1126 CDC

					Per original female		Per surviving female	
Nominal Treatment µg/L Ni	**Measured (Average) Dissolved Ni µg/L**	**Rep**	**Survival**	**total # of neonates**	**Average**	**Std Dev**	**Average**	**Std Dev**
13.2	12.3	A	1	32	35.7	3.7	35.7	3.7
13.2	12.3	B	1	38				
13.2	12.3	C	1	37				
13.2	12.3	D	1	37				
13.2	12.3	E	1	42				
13.2	12.3	F	1	35				
13.2	12.3	G	1	33				
13.2	12.3	H	1	29				
13.2	12.3	I	1	39				
13.2	12.3	J	1	35				
18.9	17.4	A	1	25	28.5	8.7	28.5	8.7
18.9	17.4	B	1	13				
18.9	17.4	C	1	36				
18.9	17.4	D	1	16				
18.9	17.4	E	1	37				
18.9	17.4	F	1	36				
18.9	17.4	G	1	29				
18.9	17.4	H	1	27				
18.9	17.4	I	1	38				
18.9	17.4	J	1	28				
26.9	23.7	A	1	11	19.0	7.6	19.7	7.8
26.9	23.7	B	1	20				
26.9	23.7	C	1	17				
26.9	23.7	D	1	32				
26.9	23.7	E	1	19				
26.9	23.7	F	1	21				
26.9	23.7	G	1	29				
26.9	23.7	H	1	21				
26.9	23.7	I	1	7				
26.9	23.7	J	**0**	**13**				
38.5	32.5	A	**0**	**5**	10.1	7.6	19.0	3.6
38.5	32.5	B	**0**	**4**				
38.5	32.5	C	**0**	**8**				
38.5	32.5	D						
38.5	32.5	E	1	15				
38.5	32.5	F	**0**	**0**				
38.5	32.5	G	1	22				
38.5	32.5	H	1	20				
38.5	32.5	I	**0**	**5**				
38.5	32.5	J	**0**	**12**				

ASC 8/2/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Gaussian Tolerance Distribution Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.5000	1.5000	0.0279	1.4208	1.5450
StdDev	0.2000	0.2000	0.03358	0.05038	0.12654
Y0	1.0000	1.0000	0.0233	0.8841	0.9960

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	30.40	26.35	35.08
20.0	26.44	21.52	32.48
10.0	24.57	18.47	32.69
5.0	23.14	15.90	33.66

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

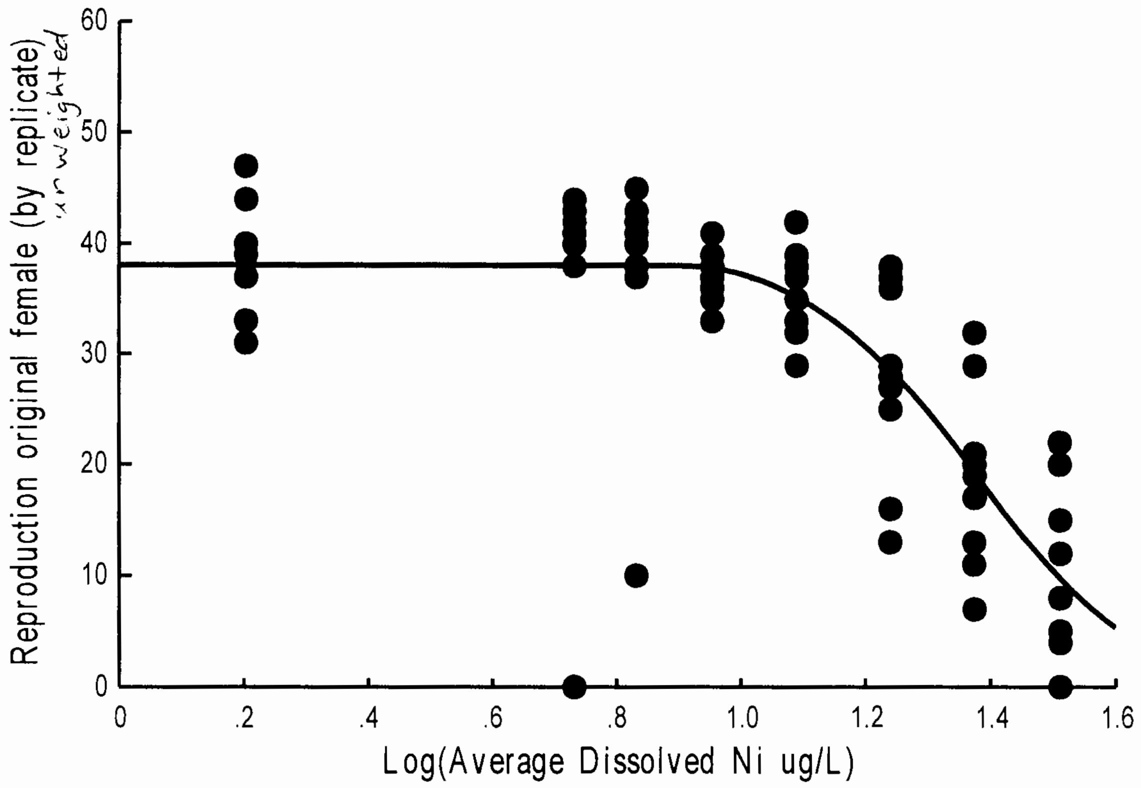
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Total N
0.2041	1.0000	0.9666	-0.0334	10.
0.7324	0.9000	0.9666	0.0666	10.
0.8325	0.9000	0.9666	0.0666	10.
0.9542	1.0000	0.9666	-0.0334	10.
1.0899	1.0000	0.9666	-0.0334	10.
1.2405	1.0000	0.9662	-0.0338	10.
1.3747	0.9000	0.9020	0.0020	10.
1.5119	0.3333	0.3324	-0.0010	9.

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3847	1.3847	0.0238	1.3313	1.4260
S	2.249	2.249	0.408	1.317	2.942
Y0	37.92	37.92	1.28	35.49	40.60

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	23.91	21.44	26.67
20.0	16.070	13.311	19.399
10.0	13.153	10.085	17.153
5.0	11.416	8.183	15.925
0.0	8.110	4.735	13.890

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	78	12146.	155.7		
Regression	2	7564.	3782.1	62.7	0.0000
Error	76	4582.	60.3		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	47.0000	38.0459	-8.9541	1.
0.2041	44.0000	38.0459	-5.9541	1.
0.2041	44.0000	38.0459	-5.9541	1.
0.2041	40.0000	38.0459	-1.9541	1.
0.2041	40.0000	38.0459	-1.9541	1.
0.2041	39.0000	38.0459	-0.9541	1.
0.2041	39.0000	38.0459	-0.9541	1.
0.2041	37.0000	38.0459	1.0459	1.
0.2041	33.0000	38.0459	5.0459	1.
0.2041	31.0000	38.0459	7.0459	1.
0.7324	44.0000	38.0459	-5.9541	1.
0.7324	43.0000	38.0459	-4.9541	1.
0.7324	43.0000	38.0459	-4.9541	1.
0.7324	42.0000	38.0459	-3.9541	1.
0.7324	42.0000	38.0459	-3.9541	1.
0.7324	41.0000	38.0459	-2.9541	1.
0.7324	40.0000	38.0459	-1.9541	1.
0.7324	38.0000	38.0459	0.0459	1.
0.7324	38.0000	38.0459	0.0459	1.
0.7324	0.0000	38.0459	38.0459	1.
0.8325	45.0000	38.0459	-6.9541	1.
0.8325	43.0000	38.0459	-4.9541	1.
0.8325	43.0000	38.0459	-4.9541	1.
0.8325	42.0000	38.0459	-3.9541	1.
0.8325	41.0000	38.0459	-2.9541	1.
0.8325	40.0000	38.0459	-1.9541	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8325	38.0000	38.0459	0.0459	1.
0.8325	38.0000	38.0459	0.0459	1.
0.8325	37.0000	38.0459	1.0459	1.
0.8325	10.0000	38.0459	28.0459	1.
0.9542	41.0000	37.8694	-3.1306	1.
0.9542	39.0000	37.8694	-1.1306	1.
0.9542	39.0000	37.8694	-1.1306	1.
0.9542	39.0000	37.8694	-1.1306	1.
0.9542	38.0000	37.8694	-0.1306	1.
0.9542	38.0000	37.8694	-0.1306	1.
0.9542	37.0000	37.8694	0.8694	1.
0.9542	36.0000	37.8694	1.8694	1.
0.9542	35.0000	37.8694	2.8694	1.
0.9542	33.0000	37.8694	4.8694	1.
1.0899	42.0000	35.2230	-6.7770	1.
1.0899	39.0000	35.2230	-3.7770	1.
1.0899	38.0000	35.2230	-2.7770	1.
1.0899	37.0000	35.2230	-1.7770	1.
1.0899	37.0000	35.2230	-1.7770	1.
1.0899	35.0000	35.2230	0.2230	1.
1.0899	35.0000	35.2230	0.2230	1.
1.0899	33.0000	35.2230	2.2230	1.
1.0899	32.0000	35.2230	3.2230	1.
1.0899	29.0000	35.2230	6.2230	1.
1.2405	38.0000	28.5637	-9.4363	1.
1.2405	37.0000	28.5637	-8.4363	1.
1.2405	36.0000	28.5637	-7.4363	1.
1.2405	36.0000	28.5637	-7.4363	1.
1.2405	29.0000	28.5637	-0.4363	1.
1.2405	28.0000	28.5637	0.5637	1.
1.2405	27.0000	28.5637	1.5637	1.
1.2405	25.0000	28.5637	3.5637	1.
1.2405	16.0000	28.5637	12.5637	1.
1.2405	13.0000	28.5637	15.5637	1.
1.3747	32.0000	19.3339	-12.6661	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

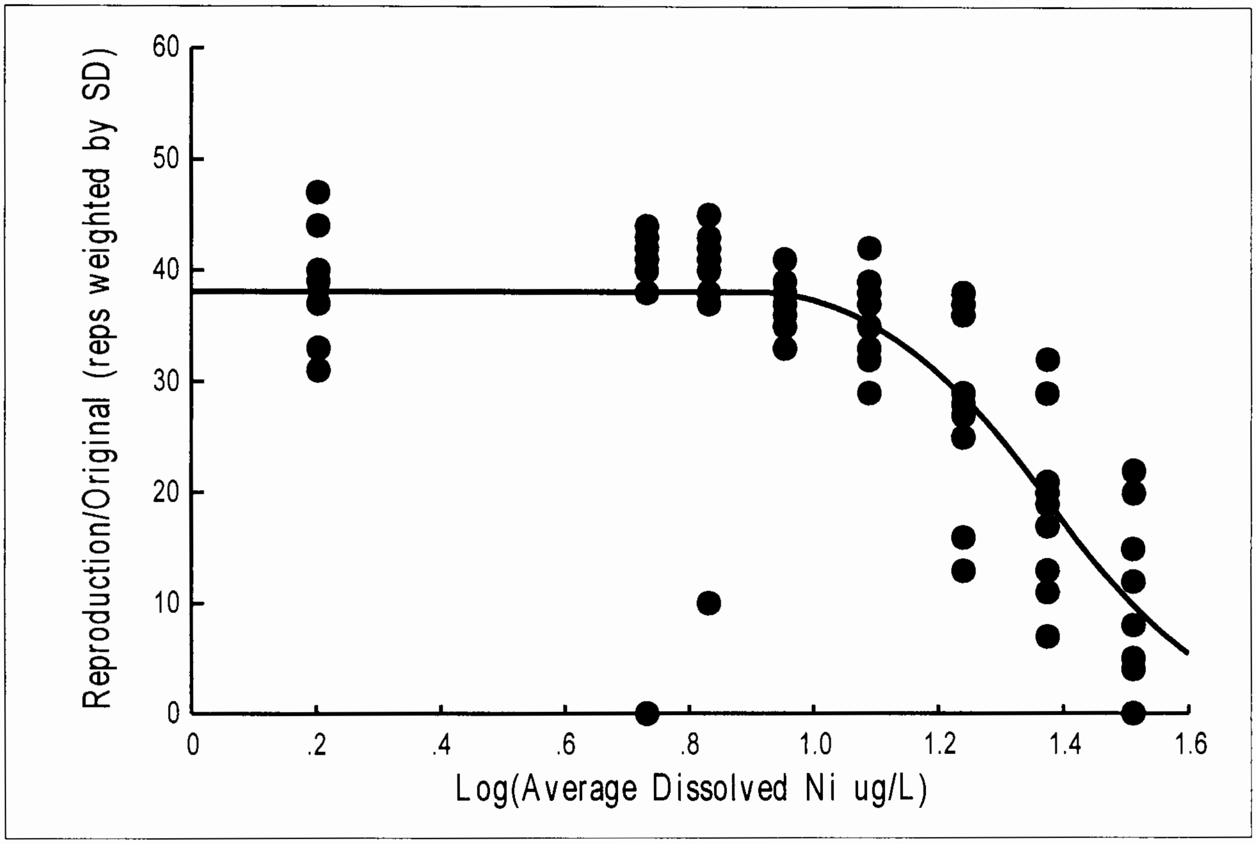
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.3747	29.0000	19.3339	-9.6661	1.
1.3747	21.0000	19.3339	-1.6661	1.
1.3747	21.0000	19.3339	-1.6661	1.
1.3747	20.0000	19.3339	-0.6661	1.
1.3747	19.0000	19.3339	0.3339	1.
1.3747	17.0000	19.3339	2.3339	1.
1.3747	13.0000	19.3339	6.3339	1.
1.3747	11.0000	19.3339	8.3339	1.
1.3747	7.0000	19.3339	12.3339	1.
1.5119	22.0000	9.7570	-12.2430	1.
1.5119	20.0000	9.7570	-10.2430	1.
1.5119	15.0000	9.7570	-5.2430	1.
1.5119	12.0000	9.7570	-2.2430	1.
1.5119	8.0000	9.7570	1.7570	1.
1.5119	5.0000	9.7570	4.7570	1.
1.5119	5.0000	9.7570	4.7570	1.
1.5119	4.0000	9.7570	5.7570	1.
1.5119	0.0000	9.7570	9.7570	1.

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.3847	1.3847	0.0217	1.3365	1.4229
S	2.249	2.249	0.310	1.510	2.746
Y0	37.92	37.92	0.83	36.40	39.72

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	23.97	21.70	26.48
20.0	16.106	13.986	18.547
10.0	13.181	10.882	15.964
5.0	11.439	9.036	14.480
0.0	8.124	5.629	11.725

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	78	68164.1	873.899		
Regression	2	68091.3	34045.629	35500.	0.0000
Error	76	72.9	0.959		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	47.0000	38.0568	-8.9432	4.9000
0.2041	44.0000	38.0568	-5.9432	4.9000
0.2041	44.0000	38.0568	-5.9432	4.9000
0.2041	40.0000	38.0568	-1.9432	4.9000
0.2041	40.0000	38.0568	-1.9432	4.9000
0.2041	39.0000	38.0568	-0.9432	4.9000
0.2041	39.0000	38.0568	-0.9432	4.9000
0.2041	37.0000	38.0568	1.0568	4.9000
0.2041	33.0000	38.0568	5.0568	4.9000
0.2041	31.0000	38.0568	7.0568	4.9000
0.7324	44.0000	38.0568	-5.9432	13.2000
0.7324	43.0000	38.0568	-4.9432	13.2000
0.7324	43.0000	38.0568	-4.9432	13.2000
0.7324	42.0000	38.0568	-3.9432	13.2000
0.7324	42.0000	38.0568	-3.9432	13.2000
0.7324	41.0000	38.0568	-2.9432	13.2000
0.7324	40.0000	38.0568	-1.9432	13.2000
0.7324	38.0000	38.0568	0.0568	13.2000
0.7324	38.0000	38.0568	0.0568	13.2000
0.7324	0.0000	38.0568	38.0568	13.2000
0.8325	45.0000	38.0568	-6.9432	10.1000
0.8325	43.0000	38.0568	-4.9432	10.1000
0.8325	43.0000	38.0568	-4.9432	10.1000
0.8325	42.0000	38.0568	-3.9432	10.1000
0.8325	41.0000	38.0568	-2.9432	10.1000
0.8325	40.0000	38.0568	-1.9432	10.1000

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8325	38.0000	38.0568	0.0568	10.1000
0.8325	38.0000	38.0568	0.0568	10.1000
0.8325	37.0000	38.0568	1.0568	10.1000
0.8325	10.0000	38.0568	28.0568	10.1000
0.9542	41.0000	37.8865	-3.1135	2.3000
0.9542	39.0000	37.8865	-1.1135	2.3000
0.9542	39.0000	37.8865	-1.1135	2.3000
0.9542	39.0000	37.8865	-1.1135	2.3000
0.9542	38.0000	37.8865	-0.1135	2.3000
0.9542	38.0000	37.8865	-0.1135	2.3000
0.9542	37.0000	37.8865	0.8865	2.3000
0.9542	36.0000	37.8865	1.8865	2.3000
0.9542	35.0000	37.8865	2.8865	2.3000
0.9542	33.0000	37.8865	4.8865	2.3000
1.0899	42.0000	35.2611	-6.7389	3.7000
1.0899	39.0000	35.2611	-3.7389	3.7000
1.0899	38.0000	35.2611	-2.7389	3.7000
1.0899	37.0000	35.2611	-1.7389	3.7000
1.0899	37.0000	35.2611	-1.7389	3.7000
1.0899	35.0000	35.2611	0.2611	3.7000
1.0899	35.0000	35.2611	0.2611	3.7000
1.0899	33.0000	35.2611	2.2611	3.7000
1.0899	32.0000	35.2611	3.2611	3.7000
1.0899	29.0000	35.2611	6.2611	3.7000
1.2405	38.0000	28.6294	-9.3706	8.7000
1.2405	37.0000	28.6294	-8.3706	8.7000
1.2405	36.0000	28.6294	-7.3706	8.7000
1.2405	36.0000	28.6294	-7.3706	8.7000
1.2405	29.0000	28.6294	-0.3706	8.7000
1.2405	28.0000	28.6294	0.6294	8.7000
1.2405	27.0000	28.6294	1.6294	8.7000
1.2405	25.0000	28.6294	3.6294	8.7000
1.2405	16.0000	28.6294	12.6294	8.7000
1.2405	13.0000	28.6294	15.6294	8.7000
1.3747	32.0000	19.4278	-12.5722	7.6000

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

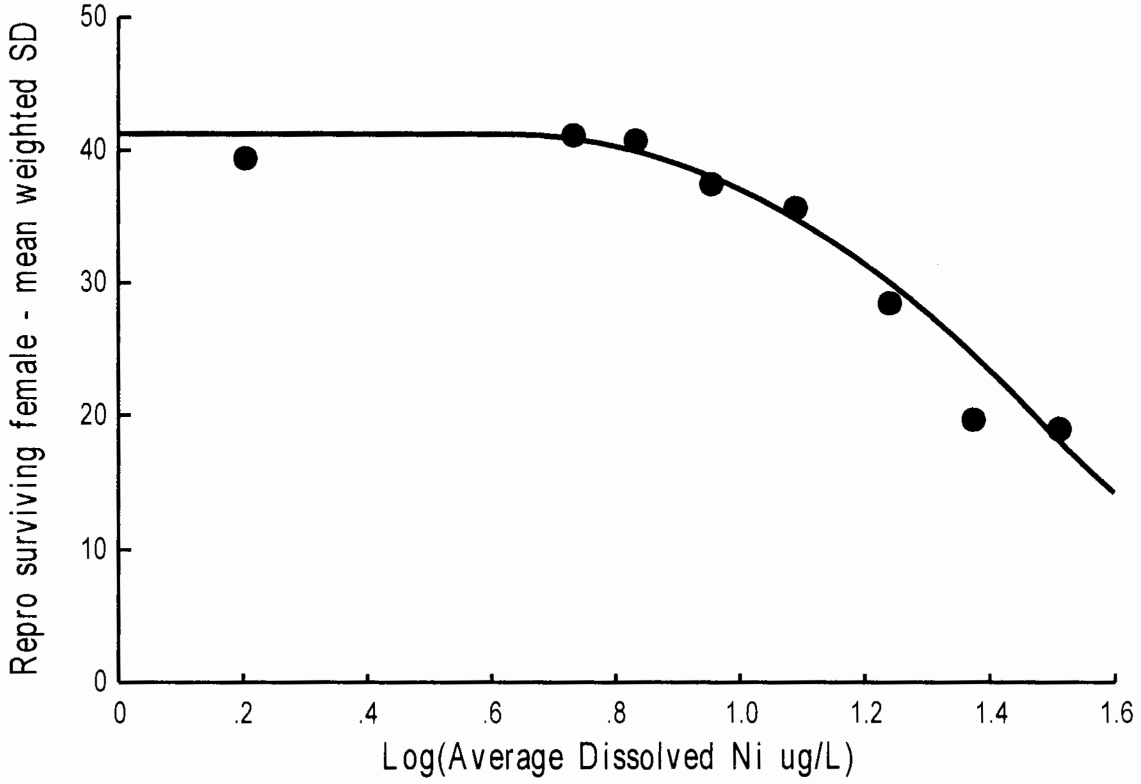
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.3747	29.0000	19.4278	-9.5722	7.6000
1.3747	21.0000	19.4278	-1.5722	7.6000
1.3747	21.0000	19.4278	-1.5722	7.6000
1.3747	20.0000	19.4278	-0.5722	7.6000
1.3747	19.0000	19.4278	0.4278	7.6000
1.3747	17.0000	19.4278	2.4278	7.6000
1.3747	13.0000	19.4278	6.4278	7.6000
1.3747	11.0000	19.4278	8.4278	7.6000
1.3747	7.0000	19.4278	12.4278	7.6000
1.5119	22.0000	9.8295	-12.1705	7.6000
1.5119	20.0000	9.8295	-10.1705	7.6000
1.5119	15.0000	9.8295	-5.1705	7.6000
1.5119	12.0000	9.8295	-2.1705	7.6000
1.5119	8.0000	9.8295	1.8295	7.6000
1.5119	5.0000	9.8295	4.8295	7.6000
1.5119	5.0000	9.8295	4.8295	7.6000
1.5119	4.0000	9.8295	5.8295	7.6000
1.5119	0.0000	9.8295	9.8295	7.6000

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4204	1.4204	0.0293	1.3843	1.5350
S	1.5039	1.5039	0.1624	0.7829	1.6178
Y0	40.47	40.47	1.19	38.20	44.33

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	28.82	24.23	34.28
20.0	14.238	10.558	19.200
10.0	9.980	6.610	15.068
5.0	7.763	4.681	12.874
0.0	4.232	1.878	9.535

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MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	7	5990.450	855.7786		
Regression	2	5989.604	2994.8018	17682.	0.0000
Error	5	0.847	0.1694		

Data Summary

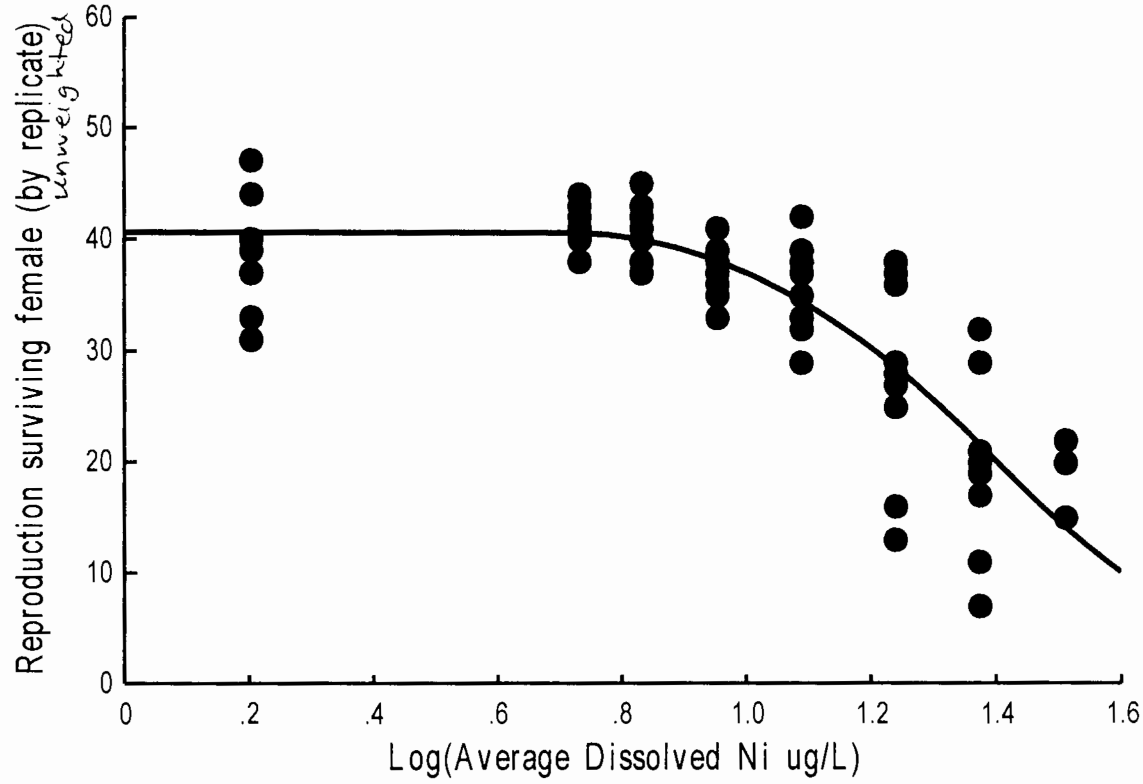
Expos Var.	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	39.4000	41.2650	1.8650	4.9000
0.7324	41.2000	40.9320	-0.2680	2.2000
0.8325	40.8000	40.0040	-0.7960	2.7000
0.9542	37.5000	38.0728	0.5728	2.3000
1.0899	35.7000	34.8826	-0.8174	3.7000
1.2405	28.5000	30.0578	1.5578	8.7000
1.3747	19.7000	24.6234	4.9234	7.8000
1.5119	19.0000	18.1261	-0.8739	3.6000

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4202	1.4202	0.0259	1.3466	1.4500
S	1.5044	1.5044	0.2269	1.0090	1.9148
Y0	40.47	40.47	1.16	38.30	42.91

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	25.02	22.21	28.18
20.0	14.024	11.650	16.882
10.0	10.475	8.008	13.703
5.0	8.522	6.092	11.922
0.0	5.179	3.092	8.673

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	69	5841.	84.7		
Regression	2	4002.	2001.2	72.9	0.0000
Error	67	1839.	27.4		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	47.0000	40.6056	-6.3944	1.
0.2041	44.0000	40.6056	-3.3944	1.
0.2041	44.0000	40.6056	-3.3944	1.
0.2041	40.0000	40.6056	0.6056	1.
0.2041	40.0000	40.6056	0.6056	1.
0.2041	39.0000	40.6056	1.6056	1.
0.2041	39.0000	40.6056	1.6056	1.
0.2041	37.0000	40.6056	3.6056	1.
0.2041	33.0000	40.6056	7.6056	1.
0.2041	31.0000	40.6056	9.6056	1.
0.7324	44.0000	40.5913	-3.4087	1.
0.7324	43.0000	40.5913	-2.4087	1.
0.7324	43.0000	40.5913	-2.4087	1.
0.7324	42.0000	40.5913	-1.4087	1.
0.7324	42.0000	40.5913	-1.4087	1.
0.7324	41.0000	40.5913	-0.4087	1.
0.7324	40.0000	40.5913	0.5913	1.
0.7324	38.0000	40.5913	2.5913	1.
0.7324	38.0000	40.5913	2.5913	1.
0.8325	45.0000	39.9988	-5.0012	1.
0.8325	43.0000	39.9988	-3.0012	1.
0.8325	43.0000	39.9988	-3.0012	1.
0.8325	42.0000	39.9988	-2.0012	1.
0.8325	41.0000	39.9988	-1.0012	1.
0.8325	40.0000	39.9988	-0.0012	1.
0.8325	38.0000	39.9988	1.9988	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8325	38.0000	39.9988	1.9988	1.
0.8325	37.0000	39.9988	2.9988	1.
0.9542	41.0000	38.1064	-2.8936	1.
0.9542	39.0000	38.1064	-0.8936	1.
0.9542	39.0000	38.1064	-0.8936	1.
0.9542	39.0000	38.1064	-0.8936	1.
0.9542	38.0000	38.1064	0.1064	1.
0.9542	38.0000	38.1064	0.1064	1.
0.9542	37.0000	38.1064	1.1064	1.
0.9542	36.0000	38.1064	2.1064	1.
0.9542	35.0000	38.1064	3.1064	1.
0.9542	33.0000	38.1064	5.1064	1.
1.0899	42.0000	34.4824	-7.5176	1.
1.0899	39.0000	34.4824	-4.5176	1.
1.0899	38.0000	34.4824	-3.5176	1.
1.0899	37.0000	34.4824	-2.5176	1.
1.0899	37.0000	34.4824	-2.5176	1.
1.0899	35.0000	34.4824	-0.5176	1.
1.0899	35.0000	34.4824	-0.5176	1.
1.0899	33.0000	34.4824	1.4824	1.
1.0899	32.0000	34.4824	2.4824	1.
1.0899	29.0000	34.4824	5.4824	1.
1.2405	38.0000	28.5868	-9.4132	1.
1.2405	37.0000	28.5868	-8.4132	1.
1.2405	36.0000	28.5868	-7.4132	1.
1.2405	36.0000	28.5868	-7.4132	1.
1.2405	29.0000	28.5868	-0.4132	1.
1.2405	28.0000	28.5868	0.5868	1.
1.2405	27.0000	28.5868	1.5868	1.
1.2405	25.0000	28.5868	3.5868	1.
1.2405	16.0000	28.5868	12.5868	1.
1.2405	13.0000	28.5868	15.5868	1.
1.3747	32.0000	21.6762	-10.3238	1.
1.3747	29.0000	21.6762	-7.3238	1.
1.3747	21.0000	21.6762	0.6762	1.

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

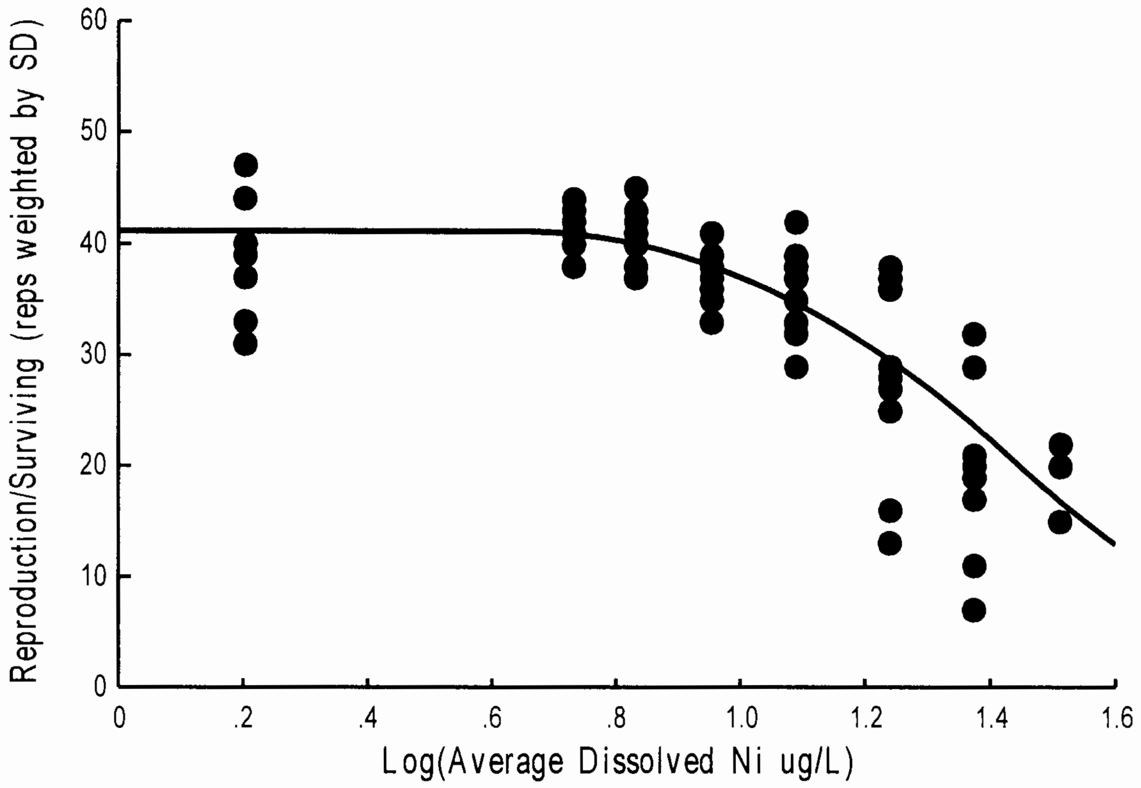
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.3747	21.0000	21.6762	0.6762	1.
1.3747	20.0000	21.6762	1.6762	1.
1.3747	19.0000	21.6762	2.6762	1.
1.3747	17.0000	21.6762	4.6762	1.
1.3747	11.0000	21.6762	10.6762	1.
1.3747	7.0000	21.6762	14.6762	1.
1.5119	22.0000	14.1197	-7.8803	1.
1.5119	20.0000	14.1197	-5.8803	1.
1.5119	15.0000	14.1197	-0.8803	1.

Error Summary

No Errors

ASC 8/1/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX50	1.4202	1.4202	0.0312	1.3755	1.5001
S	1.5044	1.5044	0.1555	0.9629	1.5837
Y0	40.47	40.47	0.89	39.35	42.92

Effect Concentration Summary

%Effect	Xp Est	95%LCL	95%UCL
50.0	27.40	23.74	31.63
20.0	14.096	11.927	16.661
10.0	10.084	8.009	12.697
5.0	7.957	5.963	10.618
0.0	4.492	2.794	7.221

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MED Toxicity Relationship Analysis Model, Version 1.30

ASC 8/2/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Regression Analysis of Variance

Source	df	SS	MS	F	Sig
Total(Adj)	69	55863.6	809.617		
Regression	2	55794.1	27897.039	26882.	0.0000
Error	67	69.5	1.038		

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.2041	47.0000	41.1340	-5.8660	4.9000
0.2041	44.0000	41.1340	-2.8660	4.9000
0.2041	44.0000	41.1340	-2.8660	4.9000
0.2041	40.0000	41.1340	1.1340	4.9000
0.2041	40.0000	41.1340	1.1340	4.9000
0.2041	39.0000	41.1340	2.1340	4.9000
0.2041	39.0000	41.1340	2.1340	4.9000
0.2041	37.0000	41.1340	4.1340	4.9000
0.2041	33.0000	41.1340	8.1340	4.9000
0.2041	31.0000	41.1340	10.1340	4.9000
0.7324	44.0000	40.9207	-3.0793	2.2000
0.7324	43.0000	40.9207	-2.0793	2.2000
0.7324	43.0000	40.9207	-2.0793	2.2000
0.7324	42.0000	40.9207	-1.0793	2.2000
0.7324	42.0000	40.9207	-1.0793	2.2000
0.7324	41.0000	40.9207	-0.0793	2.2000
0.7324	40.0000	40.9207	0.9207	2.2000
0.7324	38.0000	40.9207	2.9207	2.2000
0.7324	38.0000	40.9207	2.9207	2.2000
0.8325	45.0000	40.0524	-4.9476	2.7000
0.8325	43.0000	40.0524	-2.9476	2.7000
0.8325	43.0000	40.0524	-2.9476	2.7000
0.8325	42.0000	40.0524	-1.9476	2.7000
0.8325	41.0000	40.0524	-0.9476	2.7000
0.8325	40.0000	40.0524	0.0524	2.7000
0.8325	38.0000	40.0524	2.0524	2.7000

ASC 8/2/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8325	38.0000	40.0524	2.0524	2.7000
0.8325	37.0000	40.0524	3.0524	2.7000
0.9542	41.0000	38.0961	-2.9039	2.3000
0.9542	39.0000	38.0961	-0.9039	2.3000
0.9542	39.0000	38.0961	-0.9039	2.3000
0.9542	39.0000	38.0961	-0.9039	2.3000
0.9542	38.0000	38.0961	0.0961	2.3000
0.9542	38.0000	38.0961	0.0961	2.3000
0.9542	37.0000	38.0961	1.0961	2.3000
0.9542	36.0000	38.0961	2.0961	2.3000
0.9542	35.0000	38.0961	3.0961	2.3000
0.9542	33.0000	38.0961	5.0961	2.3000
1.0899	42.0000	34.7515	-7.2485	3.7000
1.0899	39.0000	34.7515	-4.2485	3.7000
1.0899	38.0000	34.7515	-3.2485	3.7000
1.0899	37.0000	34.7515	-2.2485	3.7000
1.0899	37.0000	34.7515	-2.2485	3.7000
1.0899	35.0000	34.7515	-0.2485	3.7000
1.0899	35.0000	34.7515	-0.2485	3.7000
1.0899	33.0000	34.7515	1.7515	3.7000
1.0899	32.0000	34.7515	2.7515	3.7000
1.0899	29.0000	34.7515	5.7515	3.7000
1.2405	38.0000	29.5994	-8.4006	8.7000
1.2405	37.0000	29.5994	-7.4006	8.7000
1.2405	36.0000	29.5994	-6.4006	8.7000
1.2405	36.0000	29.5994	-6.4006	8.7000
1.2405	29.0000	29.5994	0.5994	8.7000
1.2405	28.0000	29.5994	1.5994	8.7000
1.2405	27.0000	29.5994	2.5994	8.7000
1.2405	25.0000	29.5994	4.5994	8.7000
1.2405	16.0000	29.5994	13.5994	8.7000
1.2405	13.0000	29.5994	16.5994	8.7000
1.3747	32.0000	23.7351	-8.2649	7.8000
1.3747	29.0000	23.7351	-5.2649	7.8000
1.3747	21.0000	23.7351	2.7351	7.8000

ASC 8/2/17

Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

Data Summary

Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
1.3747	21.0000	23.7351	2.7351	7.8000
1.3747	20.0000	23.7351	3.7351	7.8000
1.3747	19.0000	23.7351	4.7351	7.8000
1.3747	17.0000	23.7351	6.7351	7.8000
1.3747	11.0000	23.7351	12.7351	7.8000
1.3747	7.0000	23.7351	16.7351	7.8000
1.5119	22.0000	16.8681	-5.1319	3.6000
1.5119	20.0000	16.8681	-3.1319	3.6000
1.5119	15.0000	16.8681	1.8681	3.6000

Error Summary

No Errors

ASC 8/2/17

APPENDIX 2 – ANCOVA ANALYSIS

An ANCOVA analysis was performed to determine if the DOC response in several datasets could be used to generate an overall DOC equation for establishing a Ni WER. The analysis was performed using data from Kozlova et al. 2009 for Ni toxicity to *Daphnia pulex* with Nordic Reservoir natural organic matter (NRNOM) additions, and Suwannee River natural organic matter (SRNOM) additions; and the data from OSU for *Ceriodaphnia dubia* with the reproductive endpoint. The Kozlova et al. 2009 data had 4 data points that were not used in this analysis with DOC ranging from 22.9 to 41.0 mg/L. These points were not used because the DOC response from the two NOM sources were inconsistent at DOC concentrations above 20 mg/L. Kozlova et al also noted that the two different NOM sources had different effects on conductivity, which suggests that ionic impurities that co-occurred with the NOM concentrates used in the experiment were different in these two samples and may relate to the different responses of these NOM sources at high concentration. Natural waters rarely have DOC concentrations above 20 mg/L, and so the effects of very high DOC concentrations on Ni toxicity are not relevant to most natural waters. Finally, the study authors have observed high DOC concentrations can lead to toxicity to aquatic organisms irrespective of the addition of a toxicant such as Ni (Chris Wood, personal communication at the SETAC NA meeting). For these reasons, and because the model does not need to be applied to DOC concentrations above 20 mg/L to be useful for the Sangamon River, data above 20 mg/L were not considered. The DOC relationship derived from this analysis should therefore be limited to DOC concentrations less than or equal to 20 mg/L. The data that were used are shown in the table below:

Table A2-0-1. DOC and Ni effect concentrations used in the ANCOVA analysis.

Group	**Measured Ni ECxx (mg/L)**	**DOC (mg/L)**
Kozlova et al. 2009 D. pulex (NRNOM)	1.47	1.53
Kozlova et al. 2009 D. pulex (NRNOM)	1.64	2.84
Kozlova et al. 2009 D. pulex (NRNOM)	3.05	9.80
Kozlova et al. 2009 D. pulex (NRNOM)	5.22	16.50
Kozlova et al. 2009 D. pulex (SRNOM)	1.00	0.50
Kozlova et al. 2009 D. pulex (SRNOM)	2.93	10.00
Kozlova et al. 2009 D. pulex (SRNOM)	2.82	19.80
OSU C. dubia reproduction	0.008	0.54
OSU C. dubia reproduction	0.016	12.20

To determine if these data had DOC slopes that were similar enough that one slope could be used for all data, an ANOCVA analysis was performed in R, the results of which are shown in the table below.

$$\log_{10} Ni = \log_{10} DOC + Group + \log_{10} DOC * Group$$

Table A2-2. Results of the ANCOVA analysis.

	df	**Sum Squares**	**Mean Square**	**F value**	**Pr(>F)**
$\log_{10}$(DOC)	1	1.2878	1.2878	229.3497	0.0006251
Group	2	7.3111	3.6555	651.0266	0.0001102
$\log_{10}$(DOC) * Group	2	0.0369	0.0184	3.2826	0.1756470
Residuals	3	0.0168	0.0056		

Because the significance of the interaction term ($\log_{10}$(DOC) * Group) is greater than 0.1, this tells us that there is no significant difference between the DOC slopes of the data from the three studies. Performing a linear regression without the interaction term, we get the following model:

$$\log_{10} Ni = 0.329 * \log_{10} DOC + Group\ intercept$$

Table A2-3. Summary of statistical results from the ANCOVA analysis.

	Estimate	**Standard Error**	**t value**	**Pr(>|t|)**
$\log_{10}$(DOC) slope	0.32914	0.05945	5.537	0.00264
Kozlova et al. 2009 D. pulex (NRNOM) intercept	0.16197	0.06690	2.421	0.06004
Kozlova et al. 2009 D. pulex (SRNOM) intercept	0.08652	0.07172	1.206	0.28165
OSU C. dubia reproduction intercept	-2.08114	0.07722	-26.950	1.32e-06

The model and data are shown in Figure A2-1. The model is significant (p = 9.95e-06) with a multiple R^2 of 0.9939.

Transcript from R:

```
> DOC.sub5[,c("group", "meas_Ni_mg.L","DOC_mg.L")]
```

```
                             group meas_Ni_mg.L DOC_mg.L
1 Kozlova et al. 2009 D. pulex (NRNOM)     1.467325     1.53
2 Kozlova et al. 2009 D. pulex (NRNOM)     1.643404     2.84
3 Kozlova et al. 2009 D. pulex (NRNOM)     3.052036     9.80
4 Kozlova et al. 2009 D. pulex (NRNOM)     5.223677    16.50
5 Kozlova et al. 2009 D. pulex (SRNOM)     0.997781     0.50
6 Kozlova et al. 2009 D. pulex (SRNOM)     2.934650    10.00
7 Kozlova et al. 2009 D. pulex (SRNOM)     2.817264    19.80
8            OSU C. dubia reproduction     0.008000     0.54
9            OSU C. dubia reproduction     0.016000    12.20
>
>
> lm.cov = lm(log10(meas_Ni_mg.L) ~ log10(DOC_mg.L) + group + log10(DOC_mg.L):group,
+             data = DOC.sub5)
> anova(lm.cov)
Analysis of Variance Table

Response: log10(meas_Ni_mg.L)
                      Df Sum Sq Mean Sq  F value    Pr(>F)
log10(DOC_mg.L)        1 1.2878  1.2878 229.3497 0.0006251 ***
group                  2 7.3111  3.6555 651.0266 0.0001102 ***
log10(DOC_mg.L):group  2 0.0369  0.0184   3.2826 0.1756470
Residuals              3 0.0168  0.0056
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
>
> lm.sub = lm(log10(meas_Ni_mg.L) ~ log10(DOC_mg.L) + group + 0,
+             data = DOC.sub5)
> summary(lm.sub) # This gives us the slope + intercepts for the groups

Call:
lm(formula = log10(meas_Ni_mg.L) ~ log10(DOC_mg.L) + group +
    0, data = DOC.sub5)

Residuals:
       1        2        3        4        5        6        7        8        9
-0.05623 -0.09543 -0.00363  0.15529  0.01159  0.05189 -0.06348  0.07231 -0.07231

Coefficients:
                                          Estimate Std. Error t value Pr(>|t|)
log10(DOC_mg.L)                            0.32914    0.05945   5.537  0.00264 **
groupKozlova et al. 2009 D. pulex (NRNOM)  0.16197    0.06690   2.421  0.06004 .
groupKozlova et al. 2009 D. pulex (SRNOM)  0.08652    0.07172   1.206  0.28165
groupOSU C. dubia reproduction            -2.08114    0.07722 -26.950 1.32e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1036 on 5 degrees of freedom
Multiple R-squared:  0.9939,     Adjusted R-squared:  0.9891
```

```
F-statistic: 205.1 on 4 and 5 DF,  p-value: 9.949e-06
```

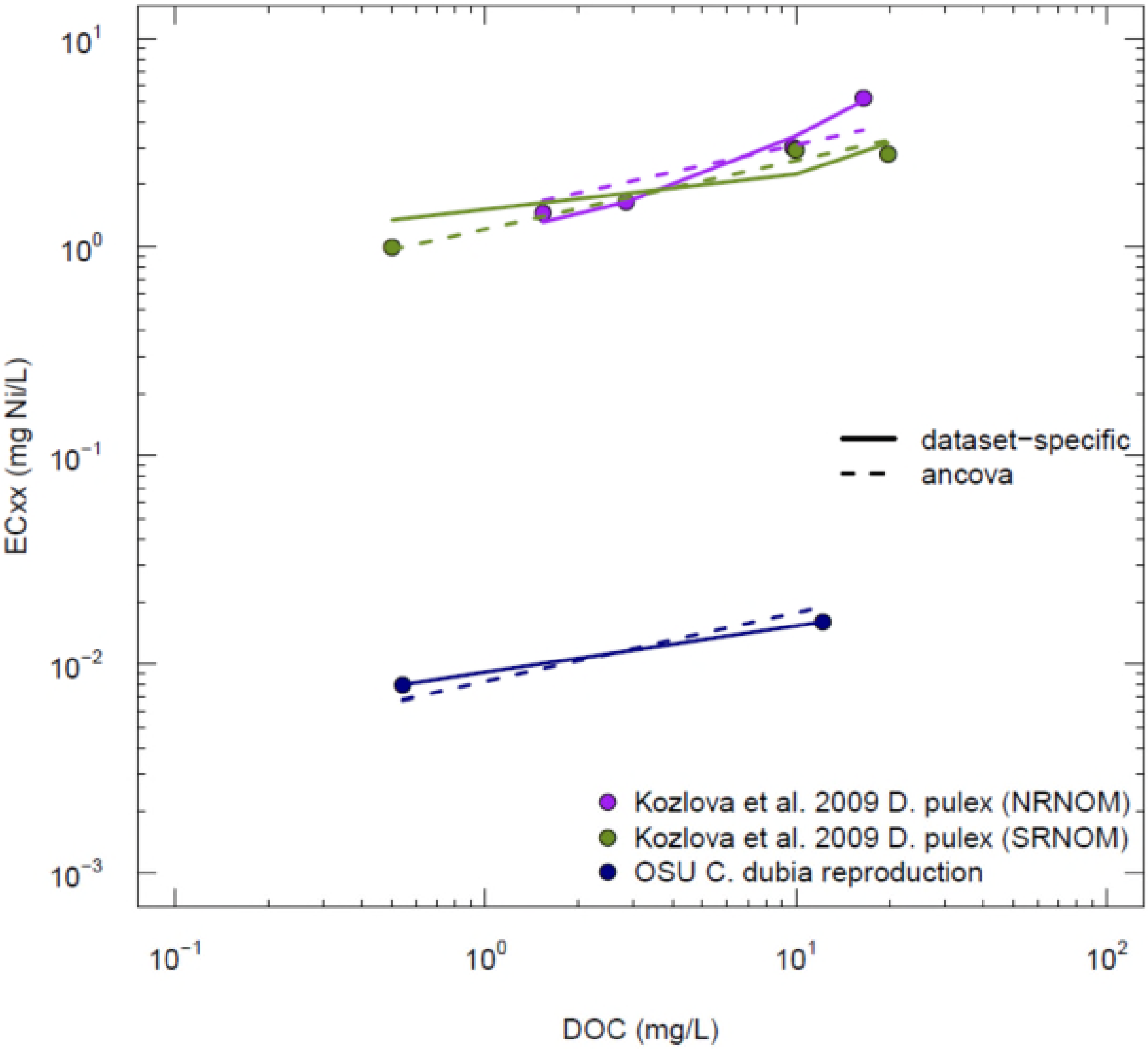

Figure A2-1. The overall best DOC regression as determined by the ANCOVA analysis is shown (dashed line) compared to the data used to develop the overall relationship (filled circles).

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Exhibit 29

Exhibit 29

Draft Manuscript

Running head: A review of water quality factors that affect nickel bioavailability to aquatic organisms: Refinement of the Biotic Ligand Model for Nickel in acute and chronic exposures

Corresponding author: Robert Santore
Windward Environmental, LLC, East Syracuse, NY 13057 USA
E-mail: RobertS@windwardenv.com
Telephone: 206-812-5450

Number of words: 9776

Robert C. Santore*, Kelly Croteau, Adam C. Ryan

Windward Environmental, LLC, 5848 Marlow Dr., East Syracuse, NY 13057 USA

* RobertS@windwardenv.com

Introduction

Nickel toxicity can be affected by a wide variety of chemical parameters, such as pH, hardness, and the presence of natural organic matter and these factors have previously been considered in the development of a Biotic Ligand Model (BLM) for Ni (Wu et al., 2003). Regulatory approaches for Ni, however, typically consider only hardness (US EPA, 1980). In order to consider a wide variety of other factors identified, including DOC, pH, and alkalinity, the Ni BLM software was developed as part of a research project for WERF (Wu et al., 2003). Since the completion of the WERF study a lot of new research has been published to investigate water quality factors that affect Ni bioavailability, and these works greatly expand the number of organisms for which bioavailability data has been generated, the range of parameter testing, and includes chronic exposures. Given the availability of these new studies, and continued interest in using the Ni BLM to assess Ni bioavailability in regulatory and risk assessment settings, this review intends to test whether a bioavailability approach based on the Ni BLM can effectively predict Ni toxicity to aquatic organisms in a wide range of conditions.

- Objectives for this work were to:
  - summarize the water quality factors that have been shown to affect nickel bioavailability and toxicity for fish and invertebrates in acute and chronic exposures,
  - evaluate the consistency of observed effects and determine if a single model can be used across different taxonomic groups, different exposure durations, and different toxicological endpoints
  - revise the nickel BLM as needed to improve the degree to which it is predictive for nickel bioavailability in a wide range of conditions in acute and chronic exposures to fish and invertebrate organisms

Model Description

The Ni BLM software was originally developed as part of a research project for WERF (Wu et al., 2003) following the development of BLM versions for Cu (Di Toro et al., 2001; Santore et al., 2001) and Zn (Santore et al., 2002). The Ni BLM shares the same overall conceptual model used for these other metals in that bioavailability is described as the interactions between factors that affect metal speciation and factors that affect metal accumulation on biological membranes (Figure 1). This shares conceptual elements with the Gill Surface Interaction Model proposed by Pagenkopf (1983) as well as the free ion activity model (Morel, 1983; Morel and Hering, 1993; Campbell, 1995). The accumulation of metals on the biotic ligand is the pathway by which toxic effects occur in organisms, often interfering with other necessary processes. The chemical speciation calculations are performed with CHESS (Santore and Driscoll, 1995), a framework that solves the system of equations associated with chemical equilibria and the charge balance. The Windermere Humic Aqueous Model (WHAM Version 1.0, Model V, Tipping 1994) is incorporated into the CHESS framework within the BLM in order to model the interactions of metals with organic matter. The use of WHAM is advantageous since it has been calibrated with a large dataset consisting of many sources of organic matter, over a wide range of chemical conditions, and for several metals including nickel.

Data Review

A literature review was performed to identify papers that reported Ni toxicity to aquatic fish and invertebrates in exposures that included a range of water chemistry. The review included studies that covered a wide variety of organisms, reported endpoints, exposure durations, and toxic effects. The studies and reported toxicity data identified in this review are summarized in Table 1. When multiple endpoints were reported in a study, the more robust endpoint was preferentially used for model comparison (e.g., EC50s were preferred over EC20, and EC20 were preferred over EC10s)

Studies selected for this review focused on variation in chemistry in synthetic and natural samples. Studies with synthetic samples used a pure water source with salt additions to design a series of conditions such that the exposure chemistry varied in a systematic way (e.g., variation in hardness) and were used in the calibration phase of model refinement. Studies with natural water samples tended to select sampling sites that provided diverse water chemical characteristics where multiple chemical parameters may co-vary from sample to sample. Studies that quantified Ni toxicity on natural water samples were used for validation of the calibrated model.

For all studies considered in this review, we required that important chemical parameters required by the BLM were measured. BLM parameters include pH, dissolved organic carbon (DOC), alkalinity, calcium, magnesium, sodium, potassium, sulfate, and chloride. Measurements for all parameters were preferred, but estimates for some parameters were acceptable if we felt that enough information was provided, or if the missing parameter was relatively unimportant. For example, if hardness was reported but little else, the estimates for the major ions were based on ion ratios calculated from another source of chemistry data with the same water body. Concentrations of DOC were only estimated in synthetic waters for which values near zero were expected. DOC was estimated as 0.3 mgC/L for acute tests and slightly higher at 0.5 mgC/L for chronic tests since feeding the organisms in the tests would contribute extra DOC. Alkalinity was estimated when necessary from pH by assuming equilibrium with atmospheric CO_2(g), such as:

$$[H_2CO_3^*] = 10^{-pCO_2} * K_H$$

$$[HCO_3^-] = \frac{K_1 * [H_2CO_3^*]}{[H^+]}$$

$$[CO_3^{2-}] = \frac{K_1 * [HCO_3^-]}{[H^+]}$$

$$Alkalinity\ (mg/L\ CaCO_3) = ([HCO_3^-] + 2 * [CO_3^{2-}] + [OH^-] - [H^+]) * \frac{100086\ mg\ CaCO_3}{2\ eq}$$

where $pCO_2 = 3.5$

$K_H = 10^{-1.5}$

$K_1 = 10^{-6.352}$

$K_2 = 10^{-10.329}$

Although the concentration of CO_2 indoors can sometimes be higher than what was used in this estimation, a validation of the alkalinity estimates compared to reported alkalinity showed that a pCO_2 of 3.5 gave an adequate prediction, and a lower value did not greatly change the estimation, nor improve the fit to measured data. All chemical inputs used in this analysis were reported in the Supplemental information (see appendix), where estimated data are identified with a bold and italic font.

Estimation method for cation and anion concentrations

Data were available for hardness, pH, and alkalinity whenever the major ions required for the BLM were not reported. Average ion ratios were used to estimate the full chemistry for each sample. The average ratios of Ca^{2+} to Mg^{2+}, Na^+, and K^+; and SO_4^{2-} to Cl^- were calculated across available data found in various literature sources (see Table 4). For this calculation, the hardness was assumed to be equivalent to Ca + Mg, so that the Ca concentration for a given sample (i) was estimated as:

$$[Ca^{2+}]_i = \frac{Hardness_i}{1 + \frac{1}{Ca\colon Mg}}$$

Estimates for the other cations (Mg^{2+}, Na^+, and K^+) were calculated by dividing the Ca ion concentration by the respective ratios, such as:

$$[Ion]_i = \frac{[Ca^{2+}]_i}{Ca\colon Ion}$$

For anions, the concentrations of bicarbonate and carbonate were estimated from pH and reported alkalinity. The remaining anions were determined to satisfy an electroneutrality condition (i.e., the sum of the equivalent charges for cations and anions must be equal). Therefore the total concentration of SO_4^{2-} and Cl^- was determined for each month as the deficit of a charge balance with the cations, pH, and alkalinity.

$$[SO_4^{2-} + Cl^-] = \ 2[Ca^{2+}] + 2[Mg^{2+}] + [Na^+] + [K^+] + [H^+] - [HCO_3^-] - 2[CO_3^{2-}] - [OH^-]$$

where $[HCO_3^-] = \frac{[Alk]-[OH^-]+[H^+]}{1+\frac{2*K_2}{[H^+]}}$

$$[CO_3^{2-}] = K_2 * \frac{[HCO_3^-]}{[H^+]}$$

$$[H^+] = 10^{-pH}$$

$$[OH^-] = 10^{-(14-pH)}$$

$$K_2 = 10^{-10.329}$$

and *Alk* = alkalinity in equivalents / L = 2 x 10^{-5} x alkalinity (as mg $CaCO_3$ / L). The concentrations of each anion were calculated using this deficit and the SO_4: Cl ion ratio:

$$[SO_4^{2-}] = \frac{[SO_4^{2-} + Cl^-]}{2 + \frac{1}{SO_4\colon Cl}}$$

$$[Cl^-] = \frac{[SO_4^{2-}]}{SO_4\colon Cl}$$

Summary of Ni bioavailability literature used in this analysis

Based on these search criteria, a number of studies were selected for model calibration and evaluation. For acute exposures, fourteen studies were selected that included toxicity data for fifteen species including fourteen invertebrates and one fish (Table 1). Chapman et al (1980) measured Ni toxicity to *D. magna* in acute exposures that varied hardness and alkalinity concentrations. Deleebeeck et al (2007a) reported toxicity data for acute exposures in synthetic water with 10 cladoceran species originating from various soft (S) or hard (H) surface waters. Individual species originating from hard surface waters were tested in moderately hard and hard water, while those originating from soft water were tested in soft and moderately hard water. Deleebeeck et al (2008) measured Ni toxicity to *D. magna* in acute exposures with synthetic and natural waters including five series of toxicity tests in synthetic waters which varied Ca, Mg, Na, pH (with $NaHCO_3$ controlling pH), and a second series with pH (with MOPS + NaOH/HCl controlling pH). Doig and Liber (2006) measured acute Ni toxicity to *H. azteca* in synthetic water containing varying amounts of DOC from multiple sources, in whole peat, peat hydrophilic DOC, humics, and fulvics forms. Hoang et al (2004) measured Ni toxicity in acute exposures using either <1 day or 28 day old fathead minnow (*P. promelas*). Individual toxicity tests in the Hoang et al (2004) study included variation in alkalinity, hardness, pH, or DOC concentrations. In addition to the data reported in 2004, additional data from a subsequent unpublished experiment are included (see Supplemental Table 5). Keithly et al (2004) performed acute exposures to measure Ni toxicity on *C.dubia* in synthetic water with variable hardness. Kozlova et al (2009) measured Ni toxicity to *D. pulex* in acute exposures with synthetic water in eight toxicity test series that varied concentrations in Ca, K, Mg, Na, DOC from the Nordic Reservoir, DOC from the Suwannee River, pH, and pH controlled by MOPS. Lind et al (1978) measured Ni toxicity in acute exposures using *D. pulicaria* and *P. promelas* in six different natural waters. Meyer et al (1999) performed acute exposures on sub-adult *P. promelas* to measure Ni toxicity at varying Ca concentrations. Pickering (1974) performed duplicate toxicity tests on 100-day-old *P. promelas* in hard and soft waters to measure acute Ni toxicity. Pyle et al (2002) measured acute Ni toxicity on larval *P. promelas* at different hardness concentrations and different pH values. Schubauer-Berigan et al (1993) measured acute Ni toxicity to *C. dubia* and *P. promelas* with variable pH.

For chronic exposures, five studies were selected with measurements from 13 species including 11 invertebrates, one fish, and one plant (Table 1). Deleebeeck et al (2007a) reported reproduction and survival chronic Ni toxicity endpoints for 9 of the 10 cladocerans (*B. coregoni* excluded) for which they also reported acute toxicity (see above). Some cladocerans showed no reproduction during the 16-21 day exposure period, and so only had a survival endpoint reported. Deleebeeck et al (2007b) measured chronic Ni toxicity on *O. mykiss* in synthetic and natural waters. Toxicity tests in synthetic waters were performed in 3 series, varying Ca, Mg, and pH concentrations. Keithly et al (2004) tested chronic Ni toxicity to *C. dubia* at varying hardness concentrations, with endpoints of reproduction and survival. In an unpublished study, Parametrix measured chronic Ni toxicity to *C. dubia* in natural and synthetic waters. Toxicity test series were performed in synthetic waters that varied alkalinity and hardness, and pH. Toxicity tests were also performed in three natural water samples, in the raw water, filtered water, and (for two of the waters) in synthetic water made to match the natural chemistry with and without DOC from that source. Schlekat et al (2010) tested chronic Ni toxicity in four natural waters, one of which was tested with and without a pH adjustment (the objective being to get a wide range of water chemistry). Toxicity tests were done on three invertebrates (*B. calyciflorus*, *C. tentans*, and *L. stagnalis*) and one plant (*L. minor*) for various endpoints.

Bioavailability Effects & Model Calibration

In the original calibration of the Ni BLM, the log K values for Ni binding to biotic ligand sites were based on measurements of Ni accumulation on the gills of *P. promelas* reported by Meyer et al., 1999. Application of the Ni BLM for this review included refinement of model parameters in recognition of the large amount of new data that have become available since the original Ni model was developed. Refinement of model parameters was performed as much as possible by considering a single parameter at a time, and comparing goodness of fit of the overall model with data that relates only to model responses to that single parameter. The parameters associated with specific water quality factors will be discussed one at a time in the sections that follow. After considering the single-parameter bioavailability experiments, the analysis was then expanded to include all data simultaneously to evaluate overall model performance in synthetic and natural waters. The ultimate goal of the model evaluation is to determine if observed Ni bioavailability factors are consistent with the conceptual model that uses chemical speciation and organism interactions to predict bioavailability, and to determine the extent to which a single set of BLM parameters could be used for all organisms in all conditions. The use of a single set of parameters in the BLM can simplify the use of model in regulatory contexts, and provides support for the interpretation of factors that affect Ni bioavailability as universal, mechanistically based processes that can be applied in a consistent way to all aquatic organisms in acute and chronic exposures. Figure 2A demonstrates this concept for the hardness effect, and Figure 2B for calcium. In these figures, the effect concentrations are normalized to the SMEA to account for the different sensitivities of different organisms. As can be seen, the effect concentrations fall closely on a single line with a significant slope, giving evidence not only that a single parameter can estimate the bioavailability effects for many organisms, but also that there is a notable hardness effect.

The goodness of fit measure used to select the calibrated Log K value was the coefficient of determination or R-squared on the logs of ECx values (logR^2). The logR^2 can be calculated as:

$$logR^2 = 1 - \frac{\sum_{i=1}^{N}(\log_{10} y_i - \log_{10} \hat{y_i})^2}{\sum_{i=1}^{N}\left(\log_{10} y_i - \overline{\log_{10} y}\right)^2} = 1 - \frac{SSE}{SST}$$

where y_i is the measured effect concentration of sample i, $\hat{y_i}$ is the predicted effect concentration, $\overline{\log_{10} y}$ is the average of the log of the measured values, SSE is the sum of square errors, and SST is the total sum of squares.

To calibrate each parameter, the data were run through the BLM in speciation mode to obtain the critical amount of Ni accumulation on the biotic ligand. Then, for each calcium, magnesium, or hardness series, the critical accumulation (CA) of the point with the minimum concentration of Ca, Mg, or hardness was selected for the toxicity run, as appropriate. The minimum value was used in calibration so that the Ca or Mg response alone were being calibrated (see Figure 3A) rather than having the slope and overall magnitude of the response change with each new log K value. The data were then run through the BLM in toxicity mode with the expert option enabled in order to get the predicted effect concentrations. These predicted values were compared to the reported values with the logR2 to select the best-fitting log K value. This procedure is repeated for Ca and Mg iteratively until the optimum log K values remain constant.

Effects of hardness on Ni toxicity

As has been widely observed previously, increasing hardness reduces Ni toxicity and bioavailability, resulting in higher effect concentrations. Figure 4 summarizes the results from acute exposures with invertebrate organisms (Figure 4A) and fish (Figure 4B). Very similar patterns of increasing Ni effect concentrations with increasing hardness are evident for invertebrates and fish. The slope of the hardness effect was typically steeper in exposures that co-varied pH and alkalinity (e.g., Chapman et al 1980), than studies that varied hardness alone (e.g. Keithly et al 2004), and this difference in expected hardness slope is consistent with BLM predictions for these studies, since experiments that vary alkalinity and pH along with hardness include the protective effects of increasing Ni-bicarbonate complexes in addition to the competition from calcium and magnesium ions that occur when hardness is varied alone.

The acute US EPA water quality criterion, also called the criterion maximum concentration or CMC, is based on a log-log equation dependent on hardness (US EPA, 1986) using an equation with the form:

$$CMC = e^{0.846[\ln(hardness)]+2.255}$$

The acute criterion is protective for most acute studies based on invertebrates, with the exception being *C. dubia* (Figure 4A) and is protective for all acute studies based on fish (Figure 4B).

The predicted BLM responses to hardness cations includes effects from both Ca and Mg and are determined by the Log K values for Ca and Mg binding to biotic ligand sites. The original Ni BLM calibration included only effects from Ca based on the limited information available at the time (Wu et al, 1986). For the present review, protective effects from both Ca and Mg are considered. To determine the appropriate log K values for Ca and Mg binding to the Ni BL site, the overall model behavior with varying log K values was compared with experimental results showing the protective effects from either cation alone or in combination.

The calibration for the calcium log K used data from Deleebeeck et al (2007b), Deleebeeck et al (2008), Kozlova et al (2009), and Meyer et al (1999) in which calcium alone was varied. Each of these studies individually calibrated to get optimum BL-Ca log K values at 3.80, 3.25, 4.50, and 4.05, respectively (see results in Figure 3B). Looking at the fit of all four studies collectively gives an optimum log K value at 4.25, which is the final log K value for the BL-Ca reaction that was decided upon. These calibrations were all done with a BL-Mg log K of 3.60.

The calibration for the magnesium log K only used data from Deleebeeck et al (2007b), Deleebeeck et al (2008), and Kozlova et al (2009), since Meyer et al (1999) did not perform a magnesium series. These three studies were calibrated to get optimum BL-Ca log K values of 3.75, 3.40, and 3.55, respectively (see results in Figure 3C). If the studies were once again calibrated collectively, the resulting optimum log K was 3.60, which was again used as the final log K for the BL-Mg reaction. These calibrations were all done with a BL-Ca log K of 4.25.

For the Ca and Mg Log Ks of the competitive interaction with Ni at BL sites were selected based on the logR2 results and correspond to 4.25 and 3.60 respectively (Table 2). The Ni BLM with these Log K values was able to predict Ni bioavailability with changing hardness conditions in acute exposures for both invertebrates (Figure 4A) and fish (Figure 4B). The same values for Ca and Mg Log Ks work well in chronic exposures based on lethal (Figure 5A) or sublethal (Figure 5B, 4C) endpoints that include variation in hardness to over 800 mg/L as $CaCO_3$.

As was noted with the acute Ni criterion, the US EPA chronic water quality criterion, also called the criterion continuous concentration or CCC, is based on a log-log equation dependent on hardness (US EPA, 1986) using an equation with the form:

$$CCC = e^{0.846[\ln(hardness)]+0.0584}$$

Both the acute and chronic US EPA criteria for Ni have the same slope for considering how the criteria should vary with hardness, and the similarity of the hardness effects in acute and chronic exposures as shown in Figure 4 (A and B) and Figure 5 (A, B, and C).

Effects of Ca on Ni toxicity

The effects of Ca alone, without co-variation in Mg or other cation concentrations, was investigated in five studies with invertebrates and fish (Figure 6). The effects of Ca on Ni toxicity in acute exposures to invertebrates were reported by Deleebeeck et al (2008) to *D. magna* and Kozlova et al (2009) to *D. pulex* (Figure 6A). In general, Ni toxicity in acute exposures to both invertebrates decreased with increasing Ca concentrations but for *D. magna* the protective effects of Ca are observed at Ca concentrations up to about 100 mg/L. At concentrations above 100 mg/L (with a corresponding hardness of 280 mg/L as $CaCO_3$) there does not appear to be any additional benefit of added Ca in the *D. magna* toxicity data reported by Deleebeeck et al (2008). For *D. pulex*, Kozlova et al (2009) reported protective effects up to about 60 mg/L.

A protective effect of increasing Ca on Ni toxicity was also reported in two studies with fish. Meyer et al (1999) reported a protective effect of Ca from 5 to about 100 mg/L in acute exposures with *P. promelas*. Deleebeeck et al (2007b) reported a protective effect of Ca from 4 to 40 mg/L in chronic exposures to *O. mykiss* and then no additional protective effect from 40 to 110 mg/L Ca.

The Ni BLM using a Log K of 4.25 for the competitive binding of Ca on Ni biotic ligand sites fit the protective response of added Ca well for most of the studies. However, for *D. magna* the protective benefit of added Ca reported by Deleebeeck et al (2008) is lower than expected based on the overall calibration to all studies (Figure 6A). In contrast, acute exposures with *D. magna* reported by Chapman et al (1980), which varied both Ca and Mg simultaneously, match the response of increasing hardness predicted by the BLM very well (Figure 4A). The two studies by Deleebeeck are the only two studies that show reduced or no protective benefit of added Ca at higher concentrations. This may be because few studies looked at Ca effects at concentrations above 100 mg/L. The only other study in this review that reported protective benefits of high concentrations of Ca was the Parametrix (unpublished) study with *C. dubia* that showed protective benefits consistent with the response predicted by the BLM to Ca concentrations of 237 mg/L. Another possible reason for the difference in observed effect of Ca on Ni toxicity seen in both of the Deleebeeck studies is that in these studies a Ca salt ($CaCl_2$) was added to soft water with a Mg concentration of around 5 mg/L to produce a wide range of Ca concentrations. As a result of this single salt addition, the Ca to Mg ratio becomes increasingly large as Ca concentrations increase. At 100 mg/L Ca and above the Ca:Mg ratio (in mg/L units) ranged from 19 to 34 in Deleebeeck et al (2008) and at 110 mg/L Ca the the Ca:Mg ratio was 37 in Deleebeeck et al (2007b). These high Ca:Mg ratios are much higher than other studies in this review, and are much than typical ratios seen in natural waters. For example, surface waters in North America typically range from 1.2 to 4.2 (10th to 90th percentile) with a median value of 2.

Deleebeeck et al (2008) noted that high concentrations of Ca introduced an additional stress in their study and for that reason the three highest Ca concentrations were excluded from subsequent analyses. An alternative explanation is that the reduced benefit of added Ca at high concentrations observed in the two studies by Deleebeeck et al (2007a, 2008) may be due to the unusual Ca:Mg ratios that resulted from the experimental design rather than the high concentrations of Ca. Other studies in this review avoided high Ca:Mg ratios either because both cations were allowed to vary to maintain a more constant ratio (Pickering 1974; Chapman et al 1980; Pyle et al, 2002; Keithly et al, 2004; Deleebeeck et al, 2007a; Parametrix – unpublished) or because variation in Ca was investigated over a smaller range of concentrations (Meyer et al 1999; Kozlova et al 2009). The possibility that the protective effects of Ca may be limited by unusual Ca:Mg ratios would explain why a consistent response to increasing Ca alone (Figure 6) or increasing Ca and Mg (Figures 2, and 4) is seen all studies included in this review where high Ca:Mg ratios were avoided.

Given that the protective effect of Ca seen in Deleebeeck et al (2008) is lower than that observed in other studies it is not surprising that the calibrated Log K of Ca binding to Ni biotic ligand sites reported by Deleebeeck et al (2008) is lower than the value suggested by this review (Table 2). The value of 4.25 used in this review is very close to the previous value used in development of the Ni BLM (Wu et al., 2003) which was based on measured Ni accumulation and measured competition between Ca and Ni in gill tissue reported by Meyer et al (1999).

Effects of Mg on Ni toxicity

The effects of Mg alone, without co-variation in Ca or other cation concentrations, was investigated in three studies with invertebrates and fish (Figure 7). The availability of studies that quantify Mg effects separately from Ca for this review is particularly useful since previous versions of the Ni BLM software included only Ca effects. In a similar experimental design as was used to investigate Ca effects, Deleebeeck et al (2008) and Kozlova et al (2009) quantified Mg effects on Ni toxicity to *D. magna* and *D. pulex* (Figure 7A) and Deleebeeck et al (2007b) quantified Mg effects on Ni toxicity to *O. mykiss* (Figure 7B).

As was noted in the Ca experiments, the organism response in the Mg experiments across these three studies showed a consistent reduction in Ni toxicity with increasing Mg at low to moderate Mg concentrations (Figure 7). The protective effect of Mg to invertebrate species reported by Deleebeeck et al (2008) and Kozlova et al (2009) were nearly identical at Mg concentrations less than 1 up to about 40 mg/L (Figure 7A), but at concentrations from 66 to 110 mg/L no additional protective effect to *D. magna* was observed. For *O. mykiss* a protective effect of Mg was observed from 3 to ~50 mg/L, but no additional protective effect was seen at concentrations above 50 mg/L (Figure 7B). For comparison the only other study found in this review that quantified Ni toxicity over this range of Mg concentrations was Parametrix (unpublished), which quantified toxicity to *C. dubia* at Mg concentrations that ranged from 3.7 to 78 mg/L. Over this range the protective effect of Mg and co-varying Ca continued to increase (Figure 5C). As was noted in the Ca experiments, the experimental manipulation of Mg without co-variation of Ca concentrations can lead to unusual Ca:Mg ratios. At the highest Mg concentrations used by both Deleebeeck et al (2008) and Deleebeeck et al (2007b) the Ca:Mg ratios were less than 0.1. Other studies in this review avoided similarly low Ca:Mg ratios by either adjusting both Ca and Mg together or by adjusting Mg over a narrower range of concentrations.

Previous versions of the Ni BLM software did not include Mg effects, since Mg was not explicitly considered in the accumulation data reported by Meyer et al (1999) used in model calibration documented by Wu et al. (2003). The value calibrated in this review, which considers the toxicity trends in the Mg-only and Mg+Ca experimental data (i.e., Figures 2 and 6) results in a Log K of 3.5 for Mg binding to the Ni BLM.

Effects of pH on Ni toxicity

The effects of pH on Ni toxicity in acute exposures were studied in three invertebrate studies and two fish studies (Figure 8). Both Deleebeeck et al (2008) and Kozlova et al (2009) investigated pH effects with and without the presence of a buffering agent. Buffering agents such as 3-morpholinepropanesulfonic acid (MOPS) are commonly used to control pH in metal toxicity studies and have been recommended for this purpose based on the fact that they do not affect metal speciation (Kandegedara and Rorabacher, 1999). Deleebeeck et al (2008) reported very little change in Ni toxicity to *D. magna* over the pH range 5.7 to 8.1, and this pattern was consistent whether or not MOPS was used in the exposures (Figure 8A). Kozlova et al (2009) also saw very little change in Ni toxicity to *D. pulex* over the pH range 5.6 to 8.3 in acute exposures without MOPS. A very different pattern, however, was reported by Kozlova et al (2009) in pH exposures with MOPS such that Ni EC50s increased with increasing pH (Figure 8A). It is unclear if the different patterns reported by these two studies are due to differences in how these two species respond to pH effects on Ni bioavailability. The fact that *D. magna* and *D. pulex* are closely related (in the same genus), and the similarity of their response to other factors compared in this review suggests that differences with respect to how MOPS may affect Ni bioavailability would be unlikely. The similarity of the reported response with and without MOPS in the tests reported by Deleebeeck et al (2008) may simply be due to the fact that MOPS was included in a relatively narrow range of pH conditions (5.7 to 6.6), whereas Kozlova et al (2009) investigated a wider range of pH conditions in tests with MOPS (i.e., 5.6 to 8.3), and the greatest differences in the pH response with and without MOPS were observed at pH values above 6.5.

Although MOPS and other pH buffers are commonly used in metal toxicity studies to help control pH, there is some controversy as to whether these compounds affect metal bioavailability. Esbaugh et al (2014) showed that the effects of changing pH on Pb toxicity to fathead minnow were different when either MOPS or enriched CO_2(g) environments were used to control pH conditions. These differences were attributed to physiological stress caused by changes in the pH gradients in apical gill membranes in fish in the presence of MOPS (Esbaugh et al., 2014). Since these buffers do not represent conditions in the natural environment, Esbaugh et al (2014) recommend that bioavailability data for metals determined in the presence of buffers should be avoided. Avoiding Ni bioavailability data in the presence of MOPS may be especially prudent since the comparison reported by Kozlova et al (2009) show inconsistent pH effects were observed in the presence of MOPS. To the extent that Ni toxicity data reported in exposures that include either MOPS or enriched CO_2(g) environments show different pH responses than natural waters or synthetic waters that more closely resemble natural conditions may be due to experimental artifacts that result from physiological stress and may not be relevant for the purpose of developing bioavailability models. Data from exposures that include either MOPS or enriched CO_2(g) environments are identified in Figure 8A and in the comments section of the supplemental data table to facilitate caution in the use of these data in subsequent model evaluations.

If only the data from Kozlova et al (2009) that do not include MOPS are considered, then there is essentially no pH effect on Ni toxicity observed for *D. pulex* over the pH range of 5.6 to 8.3, and this lack of a pH response is consistent with the predicted trend using the Ni BLM (Figure 8A) using the parameters in Table 2. The pH effect observed by Deleebeeck et al (2008) with *D. magna* showed at most a minor increase in EC50 over the pH range 5.7 to 8.1. Although the EC50s are a little higher than predicted by the Ni BLM they are still within a factor of 2 and consistent with the trend predicted by the model, which is that little if any pH effect to invertebrates is expected from pH 5.6 to 8.3.

In contrast, Schubauer-Berigan et al (1993) reported an approximately 10-fold decrease in Ni EC50s in acute exposures to *C. dubia* from pH 7.3 to 8.7 (Figure 8B). This result is unusual given the lack of a strong pH effect with other invertebrates (Figure 8A), which if anything suggested a slight increase in Ni EC50s over a much larger pH range. The experimental conditions of Schubauer-Berigan et al were also unusual in that the test chambers were sealed to prevent gas exchange after pH adjustments were made. If gas exchange was effectively prevented, these conditions would result in a CO_2(g) enriched environment in the samples with lower pH relative to the exposure at pH 8.7. It is unclear whether the unusual pH response is due to these experimental conditions, or to species-specific differences in how pH affects Ni bioavailability to *C. dubia*. The Ni BLM can be made to predict lower EC50s at high pH by adjusting the NiOH Log K from -5.5 to -4.0 (dashed line on Figure 8b), but the uniqueness of this response, the fact that is has only been observed in one sample, and the possibility that it may be due to experimental conditions, suggests that it should be replicated in other tests with *C. dubia* prior to adopting an alternate model calibration.

Schubauer-Berigan et al (1993) in acute exposures with *P. promelas* reported no pH effect over the range 7.3 to 8.7 (Figure 8C). Pyle et al (2002) also did not see a strong pH effect in acute exposures with *P. promelas*, although approximately a three-fold increase in Ni LC50s were reported over the pH range 5.5 to 8.5 (Figure 8C). The lack of a strong pH effect in acute studies with fish is consistent with the pH effects seen in all but one of the acute studies with invertebrates. The consistency of the pH response in exposures not affected by MOPS or enriched CO_2(g) environments suggests that a Ni BLM with a single set of parameters will fit both invertebrates and fish. The similarity in the observed pH response for fish and invertebrates further reinforces the notion that the unique response reported by Schubauer-Berigan et al (1993) for *C. dubia* should be replicated prior to deciding whether it should be used in the development of a bioavailability model for Ni.

Another study that showed atypical effects of pH on Ni toxicity was a chronic test with *O. mykiss* reported by Deleebeeck et al (2007b), which shows increasing Ni LC50s at pH values lower than 7.5, and with little pH effect from 7.5 to 8.5 (Figure 9). This was the only study that indicated possible proton competition at pH values below 7, and as a result the higher log K for the BL-H binding used in the original calibration (Wu et al., 2003) was a better fit than the revised value (Table 2). The BLM prediction using the lower log K for the BL-H binding value consistent with the acute tests with invertebrates and fish results in an overestimation of toxicity at low pH, compared with the with *O. mykiss* LC50s reported by Deleebeeck et al (2007b) as shown with the solid line in Figure 9. The dashed line in Figure 9 uses the higher log K for the BL-H binding (Wu et al., 2003) and more closely matches the trends in the chronic toxicity data for *O. mykiss*. However, this is the only test and only organism which suggests this level of competitive effects of protons is appropriate. It is also the only chronic test with a fish that looked at pH effects. Since the pH response seen in this study is unlike all other studies, replication of this result would be prudent, prior to recommending the higher Log K value. It should also be noted that the lowest pH exposure included MOPS buffer, and as already discussed, MOPS may alter the bioavailability of Ni and other metals.

Effects of Dissolved Organic Carbon on Ni toxicity

Three studies investigated the effects of natural organic matter (NOM) on Ni toxicity. Kozlova et al (2009) used two different organic matter sources, Suwannee River NOM (SRNOM) and Nordic Reservoir NOM NRNOM), in acute tests with *D. pulex*. Both sources of NOM, quantified by measurement of dissolved organic carbon (DOC), reduced the toxicity of Ni (Figure 10A). The effect of either type of NOM was consistent from 0 to 10 mg/L DOC showing a reduction in toxicity (higher LC50s) with higher DOC. At concentrations above 10 mg/L the effect of increasing amounts of NRNOM reduced Ni toxicity further, while SRNOM did not have an additional protective effect at concentrations above 10 mg/L. From 1 to 40 mg/L DOC, the Ni LC50s to *D. pulex* increased about 7-fold when NRNOM was added, and only about 3-fold when SRNOM was used (Figure 10A).

When similar concentrations of NOM were added to toxicity tests with a less sensitive organism, a smaller overall effect on Ni toxicity was seen. For example, Doig and Liber (2006) reported the effects of five different NOM sources on *H. azteca* including whole peat (WP), peat hydrophilic DOC (PHD), peat fulvic acid (PFA), Suwanee River fulvic acid (SRFA) and Suwanee River humic acid (SRHA). Although the range of DOC concentrations added (i.e., 0-30 mg/L) was similar to that of Kozlova et al (2009) a less than 2-fold increase in Ni LC50s was observed (Figure 10A). Doig and Liber (2006) did not see differences in the effect of NOM on Ni bioavailability and therefore concluded that the quantity of NOM was more important than the quality of the NOM.

The effects of NOM are simulated in the BLM using a set of discrete binding sites calibrated for proton and metal binding calibrated in the development of the WHAM model (Tipping 1994). The reactions developed for WHAM are simulated in the BLM as part of the overall conceptual model dealing with metal bioavailability such that NOM can bind Ni and other metals, thereby reducing the chemical activity of the metal and reducing the extent to which it can bind to biotic ligand sites (Figure 1). These reactions include metal complexation at sites with a range of binding strengths representative of different types of reactive functional groups found in NOM.

BLM simulations of the expected effects of NOM on Ni bioavailability match the overall trends observed by Kozlova et al (2009) for *D. pulex* with NRNOM addition from 1 to 35 mg/L (Figure 10A). As previously noted the effects of SRNOM addition were similar to NRNOM from 1 to 10 mg/L DOC and in this range observed effects agree well with BLM predictions. No additional protective effect was observed from SRNOM additions at DOC concentrations from 20 mg/L – 40 mg/L and in this DOC range; however the BLM predicts that additional protective effect should be expected in a manner consistent with the effects observed in the NRNOM addition (Figure 10A).

The BLM predictions for *H. azteca* match the effects of NOM additions reported by Doig and Liber (2006) over a DOC range of 0 to 35 mg/L (Figure 10A), including the observation that NOM effects to this organism are smaller than that observed for *D. pulex*. Since the BLM includes reactions with a range of NOM binding sites, complexation reactions at low metal concentrations are dominated by interactions with strong binding sites. As metal concentrations increase, the strongest binding sites become saturated with metal thereby shifting the binding of added metal to the next strongest set of binding sites. As a result, the overall strength of metal-NOM interactions is dependent on the relative concentrations of metal and NOM. As a result, the BLM predicts larger NOM effects in conditions that are associated with lower metal concentrations (e.g., more sensitive organisms or life stages) compared with conditions associated with higher metal concentrations (e.g., less sensitive organisms or life stages). This concentration-dependent behavior is illustrated by the relatively steeper slope of the BLM predicted response to NOM additions for *D. pulex* compared with *H. azteca* and is consistent with reported observations in Kozlova et al (2009) and Doig and Liber (2006) (Figure 10A).

The effects of NOM on Ni toxicity were one of several water quality parameters investigated by Hoang et al (2004) in acute exposures to *P. promelas*. Several additional toxicity tests using the same experimental design but not included in the Hoang et al (2004) study are included here (see Supplemental table). Hoang et al (2004) concluded that Ni toxicity was affected by fish age, DOC, pH, hardness, and alkalinity and those findings are consistent with the results of the Ni BLM. The Ni BLM was able to predict variation in toxicity to *P. promelas* over a wide range of pH, hardness, alkalinity, and DOC concentrations (Figure 10b).

Validation of the Ni BLM in synthetic and natural waters

Throughout this analysis, the focus has been on comparing the Ni BLM to experiments where a single water quality factor has been adjusted. Experiments where one or more water quality factors change are also useful for model evaluation. Summaries of model performance against all synthetic waters used for acute and chronic Ni toxicity tests are shown in Figure 11. The acute summary (Figure 11A) contains all of the acute studies listed in Table 1, except for pH exposures where MOPS were added and the high Ca (Ca > 100 mg/L) and high Mg (Mg > 50 mg/L) tests of Deleebeeck et al (2007b).

Throughout this analysis, the Ni BLM was applied with a consistent set of parameters including Log K values (Table 2) and species mean effect accumulations (SMEA; Table 3). The SMEAs are similar to the lethal accumulation (or LA50) term used in previous BLM modeling but this change in name is more consistent with the wide variety of lethal and sub-lethal endpoints in acute and chronic exposures used in recent BLM evaluations.

The values of SMEAs (Table 3) used in this analysis provide a measure of the sensitivity of various organisms, endpoints, and lifestages, such that lower SMEAs indicate greater sensitivity. In general, invertebrates represent the most sensitive species in both acute and chronic exposures. For organisms where information for multiple lifestages is available, such as for *P. promelas*, younger and smaller fish are more sensitive than older and larger fish and have correspondingly smaller SMEAs as a result.

The overall comparison of Ni BLM predictions for organisms in synthetic waters shows excellent agreement with nearly all predictions within a factor of two of measured values (Figure 11). Agreement within a factor of 2 has traditionally been used to indicate good performance (Di Toro et al., 2001). Recently this level of agreement was shown to correspond to the variability observed in replicate toxicity tests represented by approximately 1.5 standard deviations around the median (Santore and Ryan, 2015). This level of agreement between predicted and measure values, therefore, is comparable to the level of agreement expected between replicate measurements. One data point that falls well away from this acceptable level of agreement is the acute *C. dubia* LC50 reported by Schubauer-Berrigan at pH 8.7. Other studies that report *C. dubia* data show much better correspondence with Ni BLM predictions such as acute data from Keithly et al (2004) and chronic data from Keithly et al (2004) and Parametrix (unpublished), even when data from these other studies include observations at similar pHs. The lowest *C. dubia* LC50 reported by Schubauer-Berrigan, in this context, does seem to be anomalously low for an acute test, and is more reflective of lowest of the range of values observed in chronic *C. dubia* endpoints (Figure 11B).

Since many of the same datasets for Ni toxicity in synthetic waters used in the overall comparisons shown in Figure 11A,B were also used for calibration, this level of overall goodness of fit should be expected. Application of the Ni BLM to an independent set of toxicity tests in natural waters shows a similar level of excellent agreement in acute exposures (Figure 12A). Lind et al. (1978) measured Ni toxicity to *D. pulicaria* in samples from lakes and rivers that covered wide ranges in pH (5.8 to 8.1), DOC (2.6 to 39 mg/L), and hardness (25 to 120 mg/L as $CaCO_3$). The Ni BLM was able to accurately predict Ni toxicity over this wide range of conditions (Figure 12A), although for the three samples from Lake Superior the BLM predicted LC50s that were consistently low (see Supplemental Table). The Lake Superior samples were the highest pH conditions in the range of samples which indicate that for *D. pulicaria*, Ni toxicity may be reduced at high pH. Lind et al. (1978) also reported LC50s for *P. promelas* and for these tests the Ni BLM predicts LC50s that match measured values well including at high pH (Figure 12A). Deleebeeck et al (2008) reported Ni EC50s from eight sites in Europe that covered wide ranges in pH (5.9 to 8.1), DOC (1.8 to 26 mg/L), and hardness (13 to 266 mg/L as $CaCO_3$) to *D. magna* and the Ni BLM performed well over these wide ranges of conditions (Figure 12A).

In chronic exposures Deleebeeck et al (2007b) reported Ni LC50s for *O. mykiss* in samples from five sites. The Ni BLM performed well for all five natural waters. Although the natural waters had variation in pH from 5.6 to 8.2 there was no suggestion that the natural waters results indicated that there was a competitive interaction between Ni and protons, similar to what was seen in the synthetic waters tests from the same study (Figure 9). Parametrix (unpublished) reported IC25s for *C. dubia* in six natural waters, and while the Ni BLM predicted IC25 values close to what was measured, the model predicted higher IC25s for samples from the Grand River, which had the highest DOC (near 7.5 mg/L), even though measured IC25s at this site were among the lowest of the natural waters in this study (Figure 12B and Supplemental Table).

Schlekat et al (2010) reported chronic Ni toxicity to four different species in four natural waters. One of the natural waters (S. Platte) was tested at ambient pH and at an acidified pH which was adjusted by equilibration with an elevated CO_2(g) environment. As previously noted, elevated CO_2(g) environments may result in additional physiological stress to test organisms (Esbaugh et al 2014), and the pH adjusted samples are identified by dashed circles around individual data points on Figure 12B to allow for comparison. However, of the four species, only the *B.calyciflorus* prediction showed a large deviation from the reported value in this acidified sample. All four of these species had not previously been tested for Ni toxicity and as a result there were no previous calibrations of the Ni BLM to these organisms. Of the four species tested, the Ni BLM predicted EC20s or EC50s to *C. tentans* and *L. minor* in good agreement with measured values (Figure 12B). For *B. calyciflorus*, however, one of the five samples resulted in a large discrepancy between measured and modeled values; subsequent tests by Schlekat et al (2010) concluded that the toxicity in this sample was not due to Ni and it was removed from further consideration in their analysis. The Ni toxicity data to *L. stagnalis* reported by Schlekat et al (2010) was the only organism in this study that showed a different pattern than was expected according to the Ni BLM. Predicted toxicity in these samples deviated from observations such that predictions in high pH samples were higher than what was observed. For *L. stagnalis*, therefore, Ni toxicity may increase at high pH, making this the only dataset found in this review other than the *C. dubia* data reported by Schubaer-Berrigan that suggests increased toxicity at high pH.

Summary and conclusions

The Ni BLM using a single set of parameters was able to successfully predict the modifying effects of water chemistry on Ni bioavailability and toxicity to a wide variety of fish and invertebrates in acute and chronic exposures. Nickel toxicity was shown to be modified by a number of water quality factors including Ca, Mg, and the presence of natural organic matter and these effects were consistent for both fish and invertebrates. The consistency of these effects allowed a single set of BLM parameters (Table 2) to fit observations from a wide array of organisms in across a wide range of water chemistries in both acute and chronic tests.

The effects of pH on Ni toxicity did not appear to be consistent across all organisms. Most invertebrates and fish for which pH trends have been reported, showed very little variation in Ni bioavailability across a wide range of pH values, or a slight reduction in toxicity at pH values above 8 (Figure 8). The exceptions appear to be *O. mykiss*, *C. dubia*, and *L. stagnalis*. Tests with *O. mykiss* showed reduced toxicity at low pH in synthetic waters (Figure 9). The trend for *O. mykiss* in Figure 9 indicated that higher LC50 values were observed at low pH, and the trend leveled off from neutral pH to pH 8.5. This pattern is distinctly different from that seen in *C. dubia* which were continued to decrease at pH 8.5 and higher (Figure 8B). Hence, the *O. mykiss* trend is described as elevated at low pH, rather than reduced at high pH. However this same trend was not evident in natural water tests. For natural waters, chronic exposures with *O. mykiss* were well described by the Ni BLM over a range of pH from 5.6 to 8.2 (Figure 12B). The reason for the difference in behavior in synthetic waters and natural waters reported by Deleebeeck et al (2007b) is not clear, however the lowest pH exposure in the synthetic water series did include MOPS buffer, and other tests included in this review indicate that MOPS can affect the pH effect observed in Ni bioavailability studies (Figure 8A; Kozlova et al., 2009). Although buffers such as MOPS were designed to have no effect on metal speciation (Kandegedara and Rorabacher, 1999), they have been shown to affect metal bioavailability via alteration of the chemical microenvironment near biological membranes (Esbaugh, et al 2014). Differences observed on the effects of pH on the bioavailability of Ni in exposures with and without MOPS provide further evidence that these buffers may have unintended impacts when used in metal bioavailability studies.

Despite the differences noted in the pH trends for some organisms, the overall patterns of behavior of Ni bioavailability suggest that there are far more similarities than differences when comparing the factors that control Ni bioavailability in natural waters across wide ranges of water chemistry. These common bioavailability factors affect Ni toxicity in acute and chronic exposures to fish and invertebrate species, and suggest that a unified framework for addressing bioavailability effects such as the Ni BLM could be used to predict Ni toxicity in risk assessment and regulatory settings.

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Figure 1. Conceptual model for the Ni BLM showing interaction between chemical speciation and accumulation of Ni on biological membranes. In the Chemical Speciation box, complexation reactions between natural organic matter (NOM) or inorganic ligands such as carbonate and hydroxide can determine the amount of free Ni ion, thereby affecting the amount of Ni accumulation on biotic ligand sites (BL-Ni). Accumulation of Ni can also be affected by competition with other cations such as calcium and magnesium.

Figure 2. SMEA-normalized Ni toxicity to aquatic invertebrates (A) and fish (B) in studies that varied hardness conditions. Individual symbols correspond to reported LC50 or EC50 values. Solid lines represent BLM predicted LC50 or EC50 values for corresponding conditions.

Figure 3. Graphical representation of the effects of changes in the BL-Ca log K (A), and variation in goodness of fit statistics with Log K values for either Ca (B) or Mg (C) binding to biotic ligand sites. Goodness of fit is determined by the correlation coefficient of the log-transformed data (i.e., log-R-squared). Statistics are summarized considering only individual studies with single ion tests (i.e, Ca in A or Mg in B) or all of the studies shown combined. Numbers closer to 1 are better for log-R-Squared.

Figure 4. Acute Ni toxicity to aquatic invertebrates (A) and fish (B) in studies that varied hardness conditions. Individual symbols correspond to reported LC50 or EC50 values. Solid lines represent BLM predicted LC50 or EC50 values for corresponding conditions. The dotted line represents the US EPA acute water quality criteria.

Figure 5. Chronic Ni toxicity to aquatic invertebrates based on survival (A) or reproduction (B, C) in studies that varied hardness conditions. Individual symbols correspond to reported LC20/50 (A), EC20/50 (B), or IC25 (C) values. In panels A and B solid lines represent BLM predicted values for corresponding conditions. In panel C measured IC25 values are shown as filled circles. BLM predictions shown as "+" were run for conditions in each test, in which more than just hardness varied. The dotted line in each panel represents the US EPA chronic water quality criteria.

Figure 6. Ni toxicity to aquatic organisms in studies that varied Ca alone in acute exposures to invertebrates (A) or fish (B), or chronic exposures to fish (C). Individual symbols correspond to reported toxicity values. Solid lines represent BLM predicted values for corresponding conditions.

Figure 7. Nickel toxicity to aquatic organisms in studies that varied Mg alone in acute exposures to invertebrates (A) or fish (B). Individual symbols correspond to reported toxicity values. Solid lines represent BLM predicted values for corresponding conditions.

Figure 8. Nickel toxicity to aquatic organisms in studies that varied pH in acute exposures to invertebrates (A, B) or fish (C). Individual symbols correspond to reported toxicity values. Solid lines represent BLM predicted values for corresponding conditions. The dashed line in B shows an alternative calibration to C. dubia that emphasizes increased bioavailability at high pH.

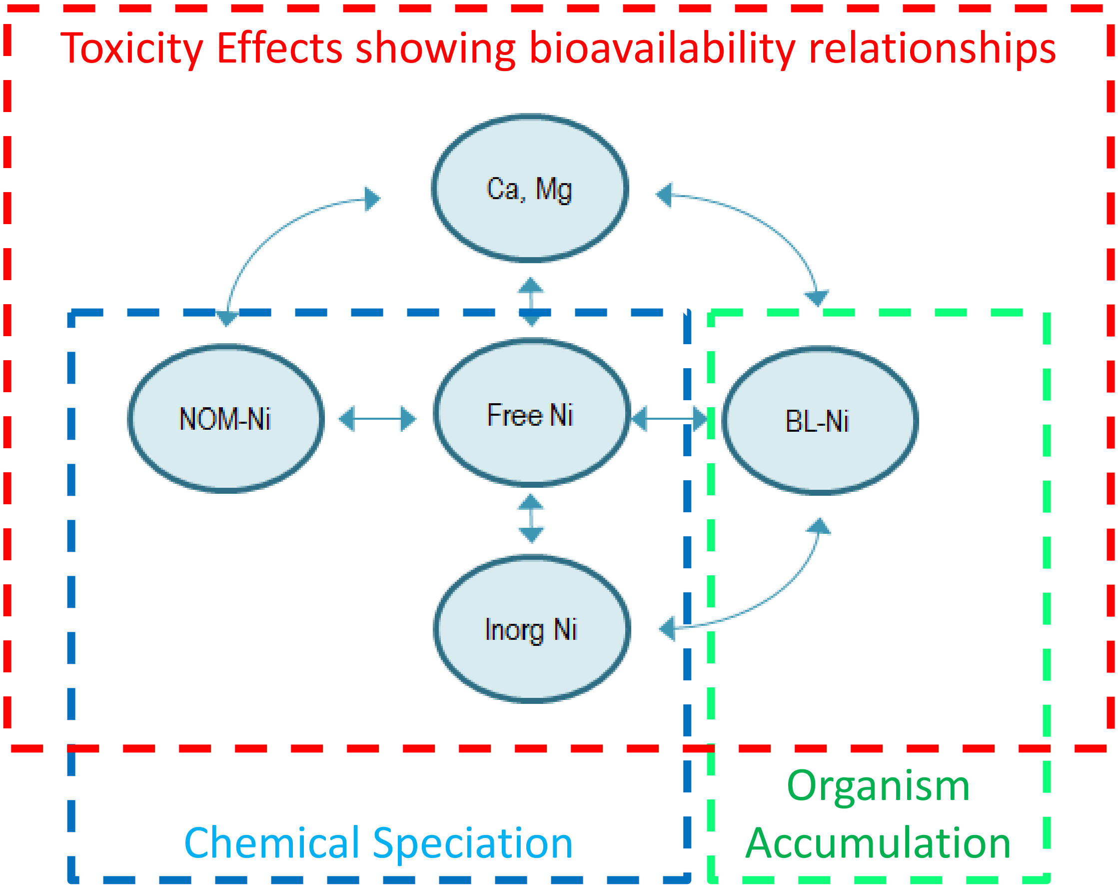
Figure 9. Nickel toxicity to aquatic organisms in studies that varied pH in chronic exposures to fish . Individual symbols correspond to reported toxicity values. Solid lines represent BLM predicted values for

corresponding conditions. The dashed line shows an alternative calibration to O. mykiss that emphasizes reduced bioavailability at low pH.

Figure 10. Nickel toxicity to aquatic organisms in studies that varied DOC in acute exposures to invertebrates (A) or fish (B). Individual symbols correspond to reported toxicity values. Solid lines represent BLM predicted values for corresponding conditions.

Figure 11. Overall performance with the best overall model for acute (A) or chronic (B) fish and invertebrate tests in synthetic waters. The solid black line shows perfect agreement between measured and predicted Ni toxicity and the dashed lines indicate plus or minus a factor of two away from perfect agreement.

Figure 12. Overall performance with the best overall model for acute (A) or chronic (B) fish and invertebrate tests in natural waters. The solid black line shows perfect agreement between measured and predicted Ni toxicity and the dashed lines indicate plus or minus a factor of two away from perfect agreement.



Toxicity Effects showing bioavailability relationships
Ca, Mg
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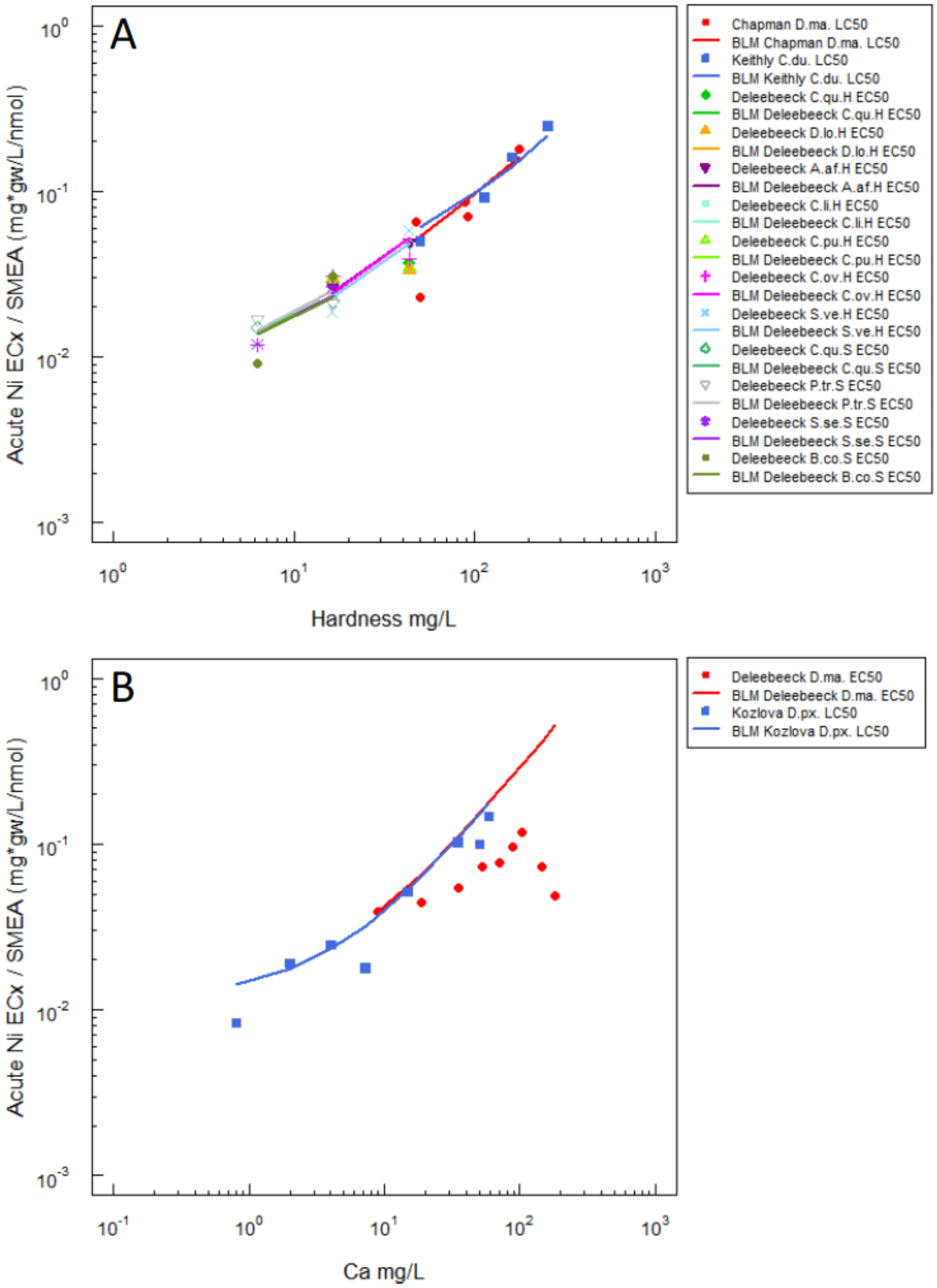
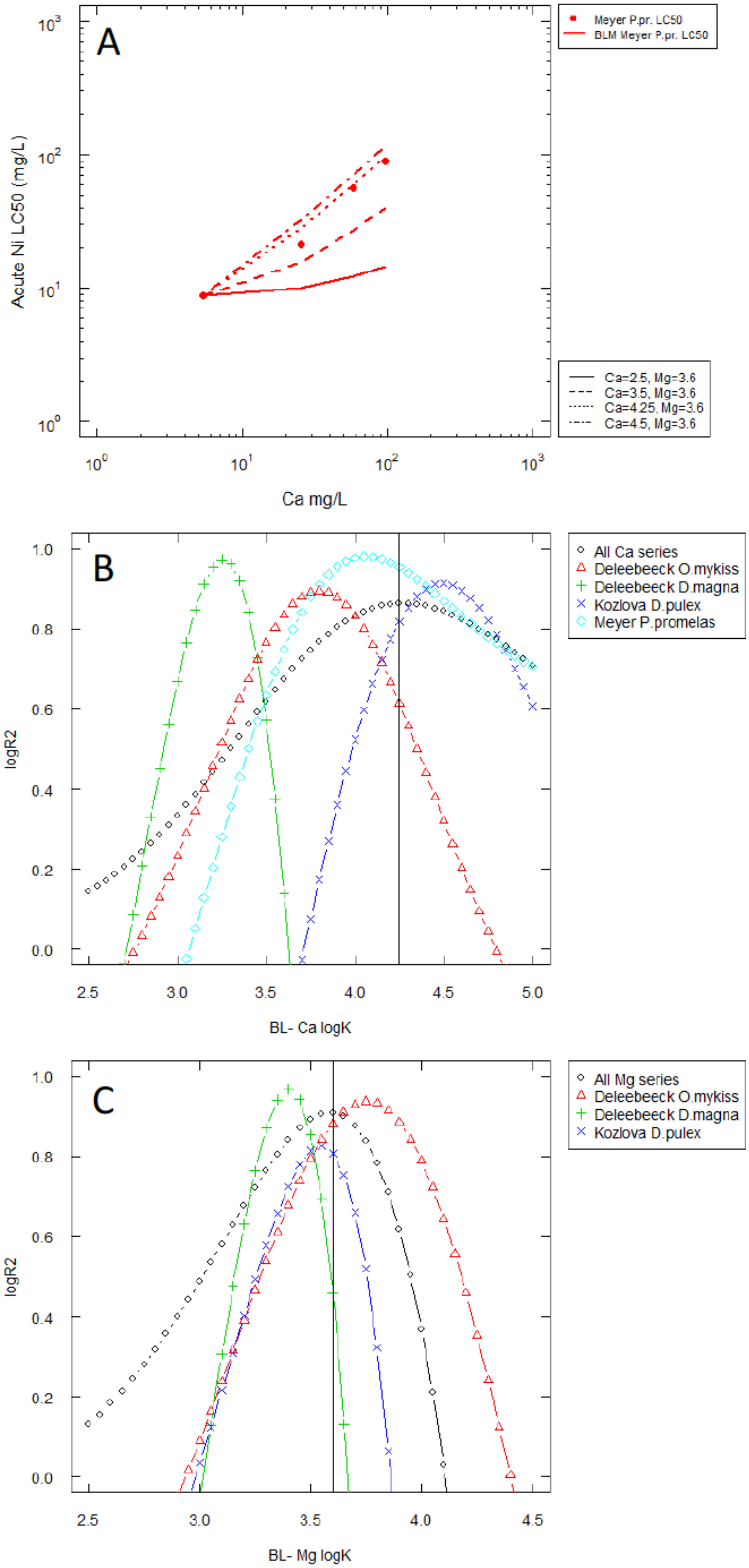
Free Ni
BL-Ni
Inorg Ni
Chemical Speciation
Organism
Accumulation


A
Acute Ni ECx / SMEA (mg*gw/L/nmol)
10^0
10^{-1}
10^{-2}
10^{-3}
10^0
10^1
10^2
10^3
Hardness mg/L
Chapman D.ma. LC50
BLM Chapman D.ma. LC50
Keithly C.du. LC50
BLM Keithly C.du. LC50
Deleebeeck C.qu.H EC50
BLM Deleebeeck C.qu.H EC50
Deleebeeck D.lo.H EC50
BLM Deleebeeck D.lo.H EC50
Deleebeeck A.af.H EC50
BLM Deleebeeck A.af.H EC50
Deleebeeck C.li.H EC50
BLM Deleebeeck C.li.H EC50
Deleebeeck C.pu.H EC50
BLM Deleebeeck C.pu.H EC50
Deleebeeck C.ov.H EC50
BLM Deleebeeck C.ov.H EC50
Deleebeeck S.ve.H EC50
BLM Deleebeeck S.ve.H EC50
Deleebeeck C.qu.S EC50
BLM Deleebeeck C.qu.S EC50
Deleebeeck P.tr.S EC50
BLM Deleebeeck P.tr.S EC50
Deleebeeck S.se.S EC50
BLM Deleebeeck S.se.S EC50
Deleebeeck B.co.S EC50
BLM Deleebeeck B.co.S EC50
B
Acute Ni ECx / SMEA (mg*gw/L/nmol)
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-1}
10^0
10^1
10^2
10^3
Ca mg/L
Deleebeeck D.ma. EC50
BLM Deleebeeck D.ma. EC50
Kozlova D.px. LC50
BLM Kozlova D.px. LC50


A
Acute Ni LC50 (mg/L)
10^3
10^2
10^1
10^0
Ca mg/L
10^0
10^1
10^2
10^3
Meyer P.pr. LC50
BLM Meyer P.pr. LC50
Ca=2.5, Mg=3.6
Ca=3.5, Mg=3.6
Ca=4.25, Mg=3.6
Ca=4.5, Mg=3.6
B
logR2
1.0
0.8
0.6
0.4
0.2
0.0
2.5
3.0
3.5
4.0
4.5
5.0
BL- Ca logK
All Ca series
Deleebeeck O.mykiss
Deleebeeck D.magna
Kozlova D.pulex
Meyer P.promelas
C
logR2
1.0
0.8
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0.4
0.2
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BL- Mg logK
All Mg series
Deleebeeck O.mykiss
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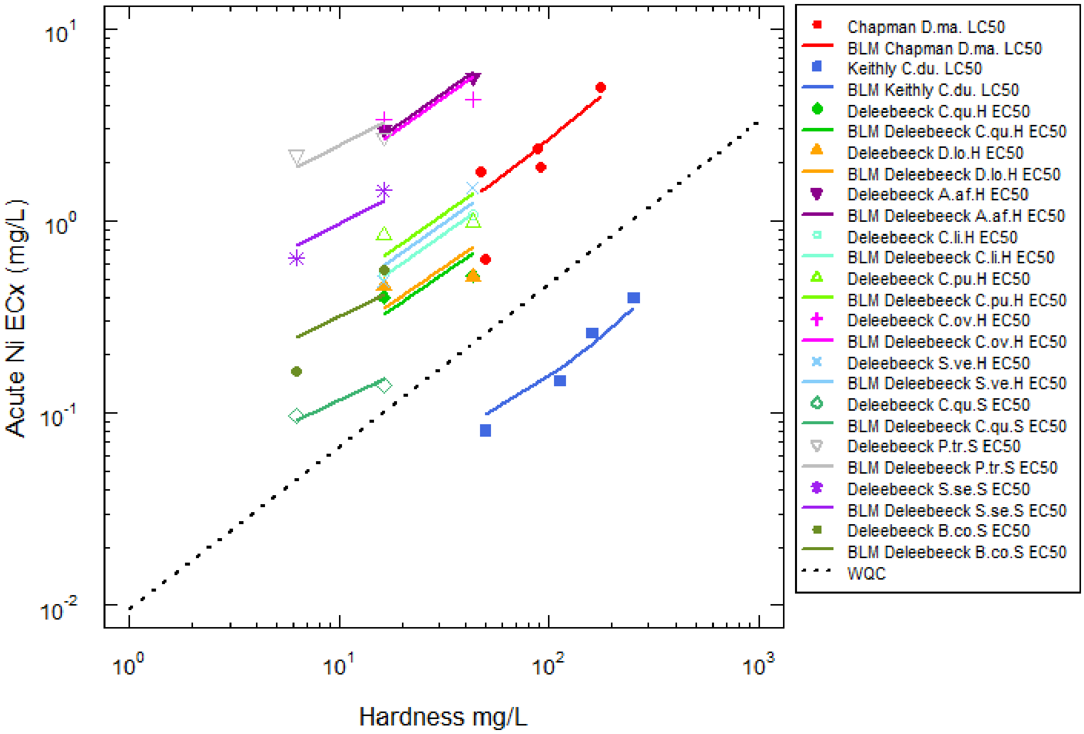
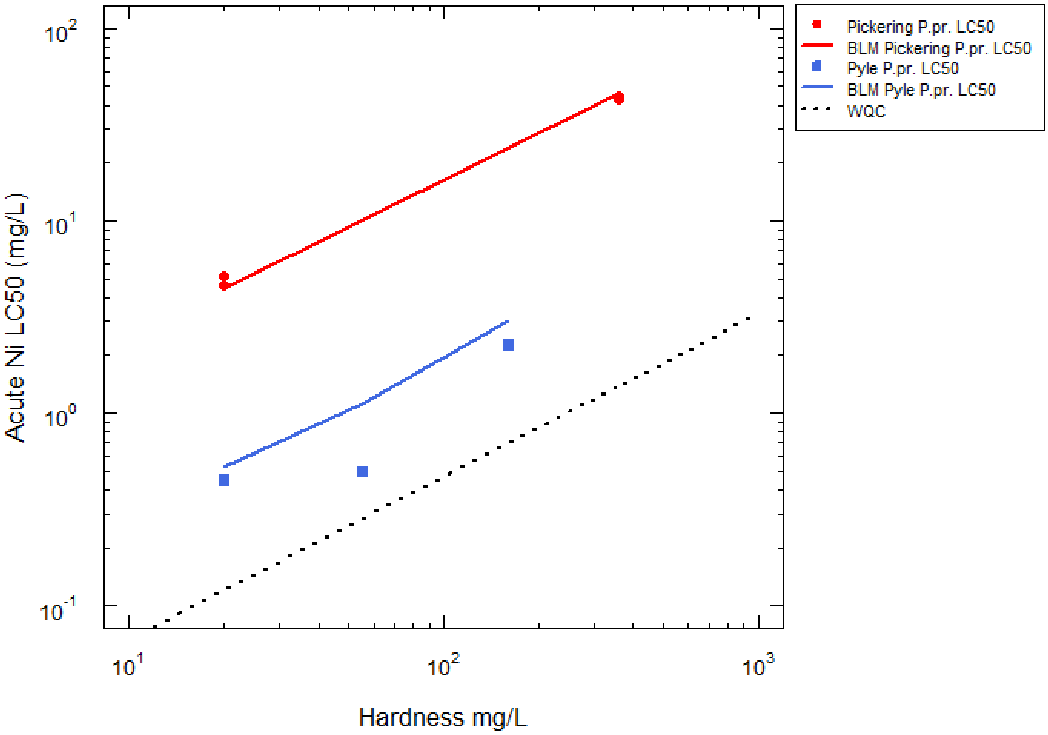
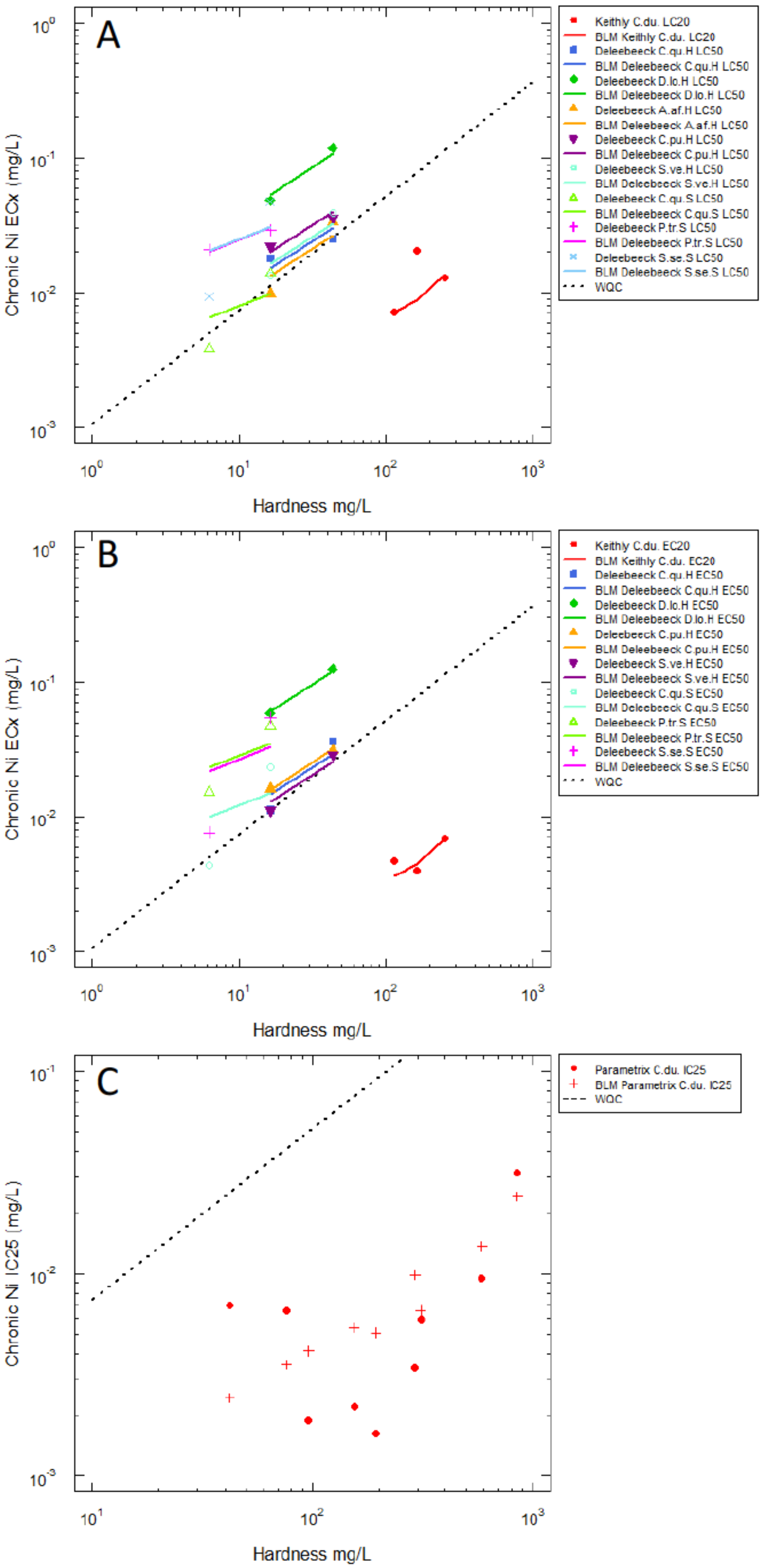
Kozlova D.pulex

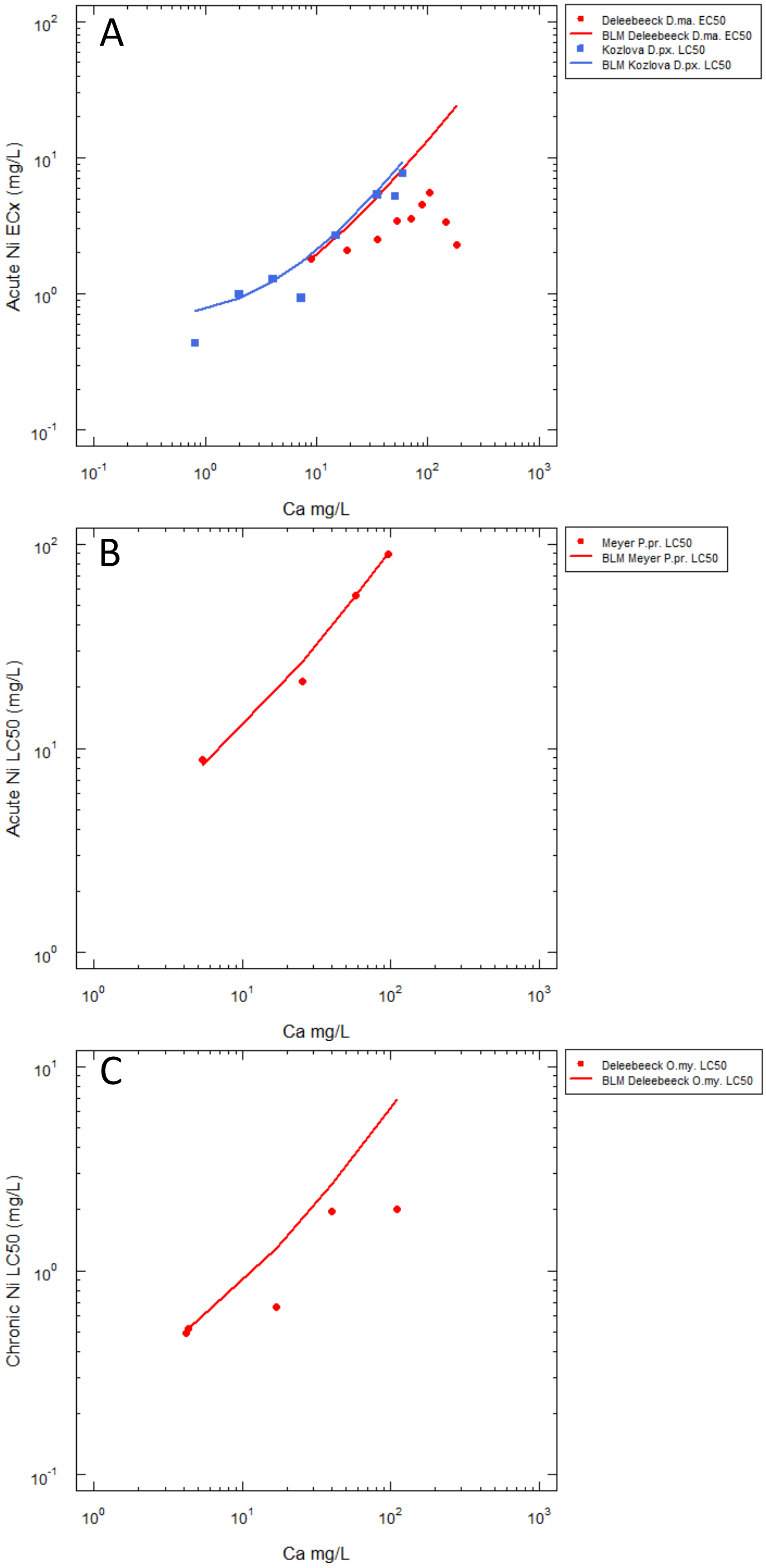
Figure


Acute Ni ECx (mg/L)
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{0}
10^{1}
10^{2}
10^{3}
Hardness mg/L
Chapman D.ma. LC50
BLM Chapman D.ma. LC50
Keithly C.du. LC50
BLM Keithly C.du. LC50
Deleebeeck C.qu.H EC50
BLM Deleebeeck C.qu.H EC50
Deleebeeck D.lo.H EC50
BLM Deleebeeck D.lo.H EC50
Deleebeeck A.af.H EC50
BLM Deleebeeck A.af.H EC50
Deleebeeck C.li.H EC50
BLM Deleebeeck C.li.H EC50
Deleebeeck C.pu.H EC50
BLM Deleebeeck C.pu.H EC50
Deleebeeck C.ov.H EC50
BLM Deleebeeck C.ov.H EC50
Deleebeeck S.ve.H EC50
BLM Deleebeeck S.ve.H EC50
Deleebeeck C.qu.S EC50
BLM Deleebeeck C.qu.S EC50
Deleebeeck P.tr.S EC50
BLM Deleebeeck P.tr.S EC50
Deleebeeck S.se.S EC50
BLM Deleebeeck S.se.S EC50
Deleebeeck B.co.S EC50
BLM Deleebeeck B.co.S EC50
WQC

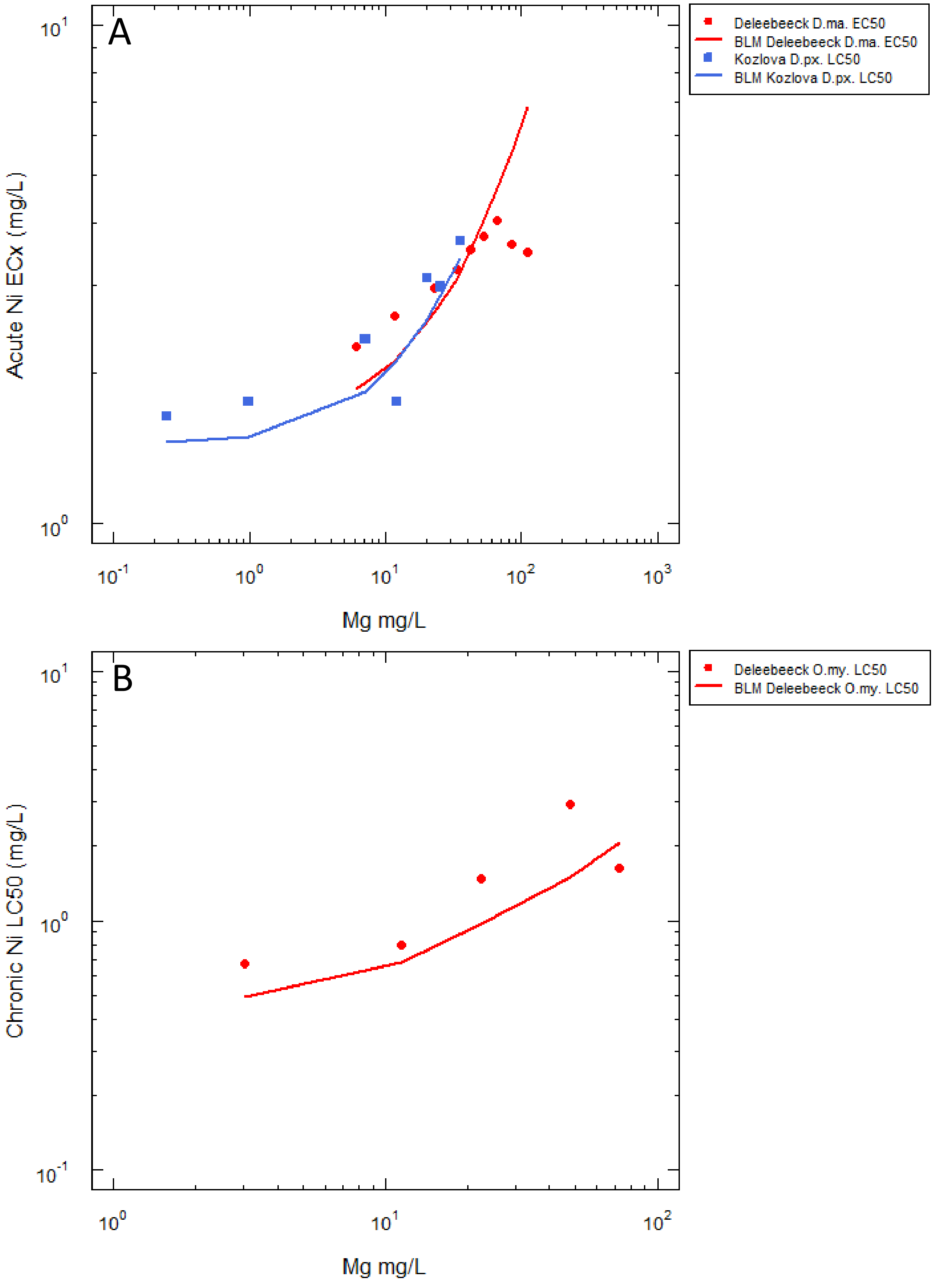

Acute Ni LC50 (mg/L)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{1}
10^{2}
10^{3}
Hardness mg/L
Pickering P.pr. LC50
BLM Pickering P.pr. LC50
Pyle P.pr. LC50
BLM Pyle P.pr. LC50
WQC

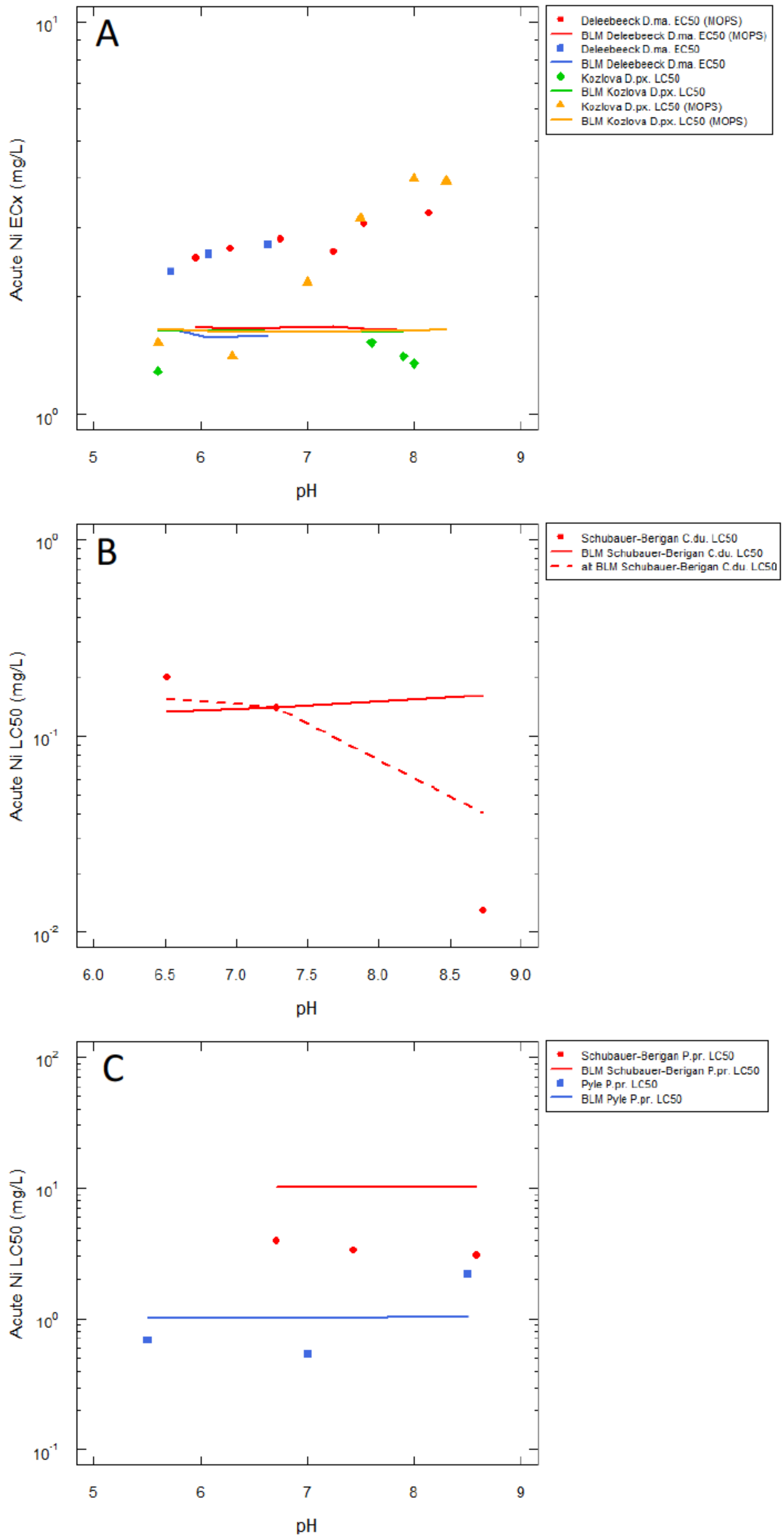

A
Chronic Ni ECx (mg/L)
10^0
10^{-1}
10^{-2}
10^{-3}
10^0
10^1
10^2
10^3
Hardness mg/L
Keithly C.du. LC20
BLM Keithly C.du. LC20
Deleebeeck C.qu.H LC50
BLM Deleebeeck C.qu.H LC50
Deleebeeck D.lo.H LC50
BLM Deleebeeck D.lo.H LC50
Deleebeeck A.af.H LC50
BLM Deleebeeck A.af.H LC50
Deleebeeck C.pu.H LC50
BLM Deleebeeck C.pu.H LC50
Deleebeeck S.ve.H LC50
BLM Deleebeeck S.ve.H LC50
Deleebeeck C.qu.S LC50
BLM Deleebeeck C.qu.S LC50
Deleebeeck P.tr.S LC50
BLM Deleebeeck P.tr.S LC50
Deleebeeck S.se.S LC50
BLM Deleebeeck S.se.S LC50
WQC
B
Chronic Ni ECx (mg/L)
10^0
10^{-1}
10^{-2}
10^{-3}
10^0
10^1
10^2
10^3
Hardness mg/L
Keithly C.du. EC20
BLM Keithly C.du. EC20
Deleebeeck C.qu.H EC50
BLM Deleebeeck C.qu.H EC50
Deleebeeck D.lo.H EC50
BLM Deleebeeck D.lo.H EC50
Deleebeeck C.pu.H EC50
BLM Deleebeeck C.pu.H EC50
Deleebeeck S.ve.H EC50
BLM Deleebeeck S.ve.H EC50
Deleebeeck C.qu.S EC50
BLM Deleebeeck C.qu.S EC50
Deleebeeck P.tr.S EC50
BLM Deleebeeck P.tr.S EC50
Deleebeeck S.se.S EC50
BLM Deleebeeck S.se.S EC50
WQC
C
Chronic Ni IC25 (mg/L)
10^{-1}
10^{-2}
10^{-3}
10^1
10^2
10^3
Hardness mg/L
Parametrix C.du. IC25
BLM Parametrix C.du. IC25
WQC

Figure

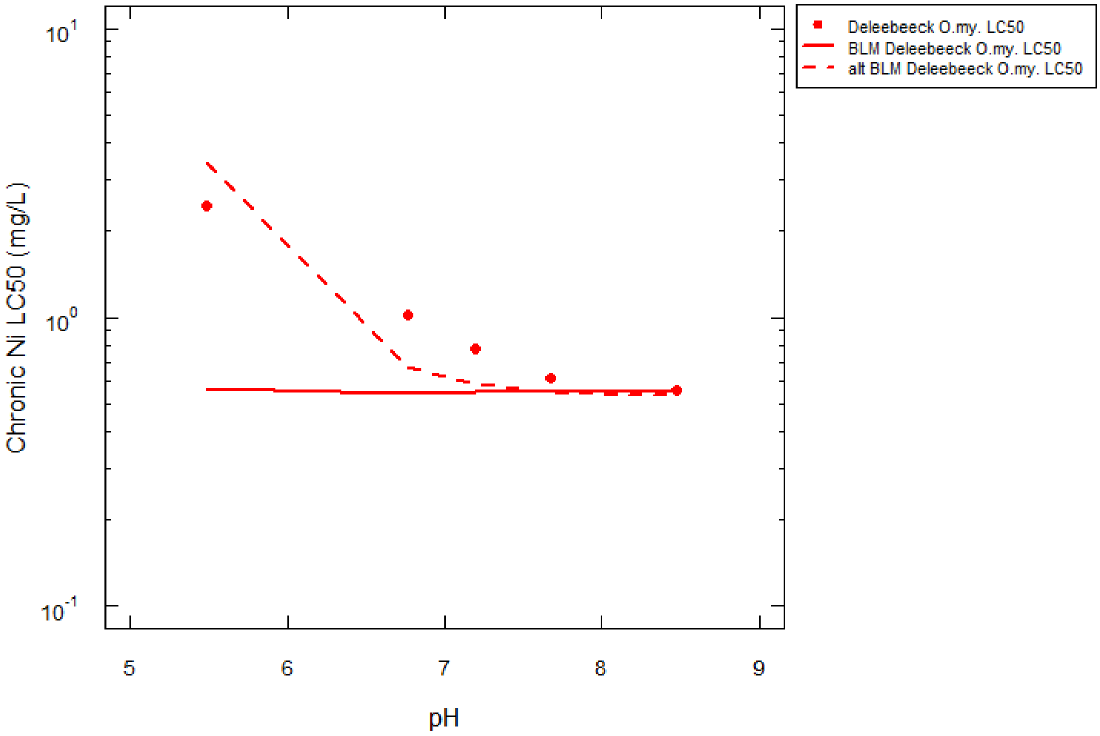

A
Acute Ni ECx (mg/L)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Ca mg/L
Deleebeeck D.ma. EC50
BLM Deleebeeck D.ma. EC50
Kozlova D.px. LC50
BLM Kozlova D.px. LC50
B
Acute Ni LC50 (mg/L)
10^{2}
10^{1}
10^{0}
10^{0}
10^{1}
10^{2}
10^{3}
Ca mg/L
Meyer P.pr. LC50
BLM Meyer P.pr. LC50
C
Chronic Ni LC50 (mg/L)
10^{1}
10^{0}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Ca mg/L
Deleebeeck O.my. LC50
BLM Deleebeeck O.my. LC50

Figure

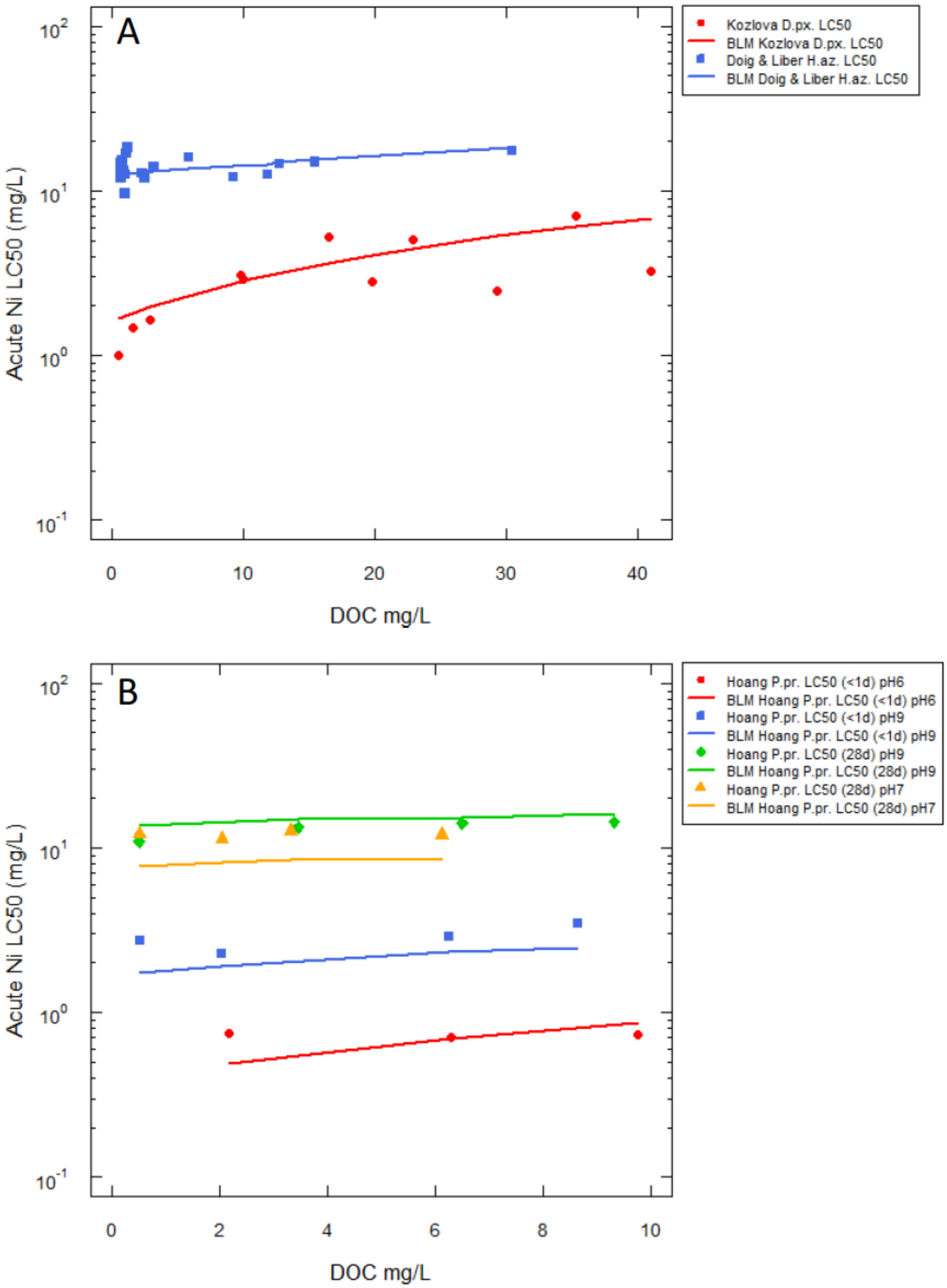
A
Acute Ni ECx (mg/L)
10^1
10^0
10^-1
10^0
10^1
10^2
10^3
Mg mg/L
Deleebeeck D.ma. EC50
BLM Deleebeeck D.ma. EC50
Kozlova D.px. LC50
BLM Kozlova D.px. LC50
B
Chronic Ni LC50 (mg/L)
10^1
10^0
10^-1
10^0
10^1
10^2
Mg mg/L
Deleebeeck O.my. LC50
BLM Deleebeeck O.my. LC50

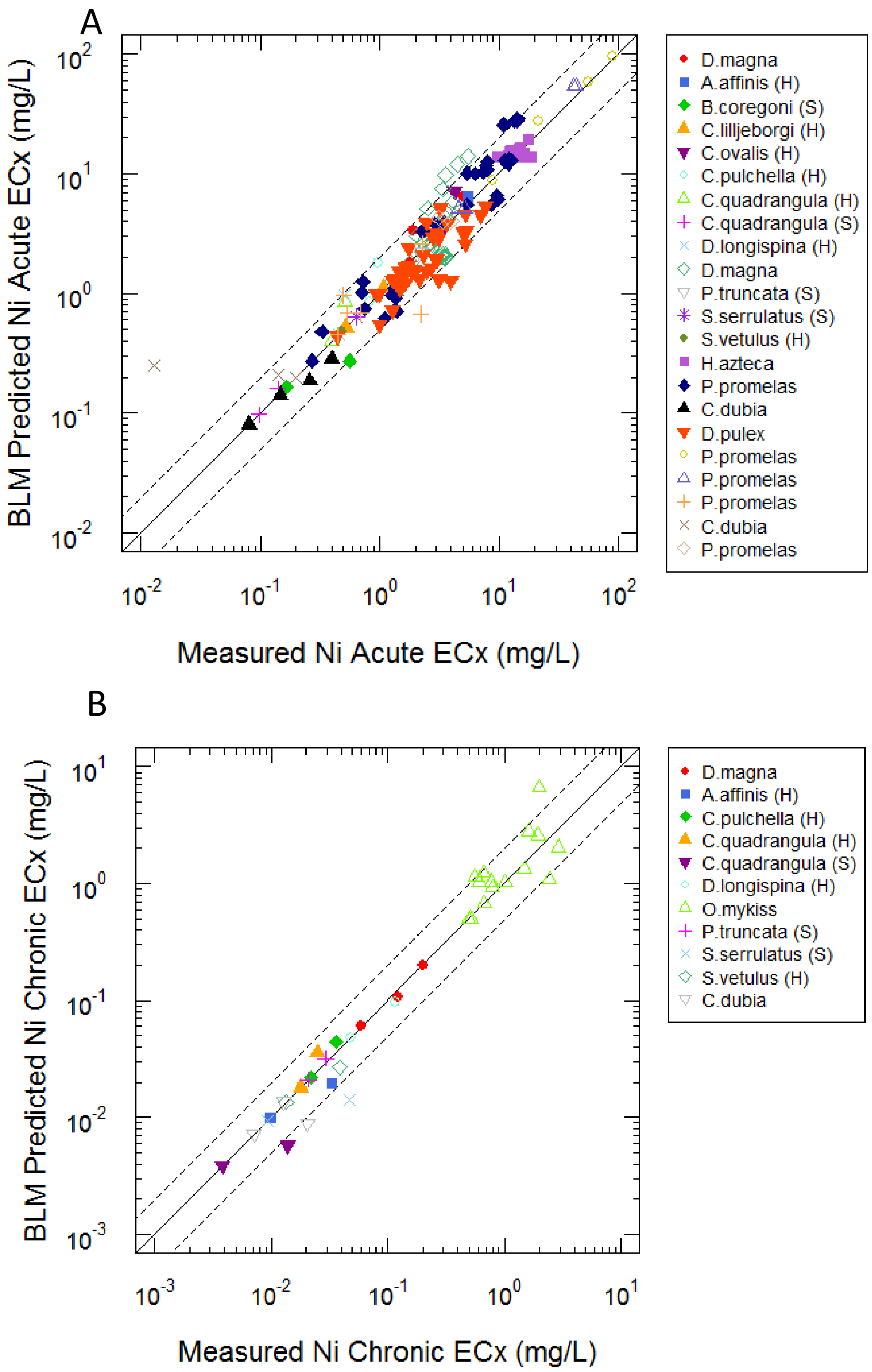

A
Acute Ni ECx (mg/L)
10^1
10^0
5
6
7
8
9
pH
Deleebeeck D.ma. EC50 (MOPS)
BLM Deleebeeck D.ma. EC50 (MOPS)
Deleebeeck D.ma. EC50
BLM Deleebeeck D.ma. EC50
Kozlova D.px. LC50
BLM Kozlova D.px. LC50
Kozlova D.px. LC50 (MOPS)
BLM Kozlova D.px. LC50 (MOPS)
B
Acute Ni LC50 (mg/L)
10^0
10^{-1}
10^{-2}
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH
Schubauer-Bergan C.du. LC50
BLM Schubauer-Berigan C.du. LC50
alt BLM Schubauer-Berigan C.du. LC50
C
Acute Ni LC50 (mg/L)
10^2
10^1
10^0
10^{-1}
5
6
7
8
9
pH
Schubauer-Bergan P.pr. LC50
BLM Schubauer-Berigan P.pr. LC50
Pyle P.pr. LC50
BLM Pyle P.pr. LC50

Figure



Chronic Ni LC50 (mg/L)
10^1
10^0
10^{-1}
5
6
7
8
9
pH
Deleebeeck O.my. LC50
BLM Deleebeeck O.my. LC50
alt BLM Deleebeeck O.my. LC50


A
Acute Ni LC50 (mg/L)
10^2
10^1
10^0
10^{-1}
0
10
20
30
40
DOC mg/L
Kozlova D.px. LC50
BLM Kozlova D.px. LC50
Doig & Liber H.az. LC50
BLM Doig & Liber H.az. LC50
B
Acute Ni LC50 (mg/L)
10^2
10^1
10^0
10^{-1}
0
2
4
6
8
10
DOC mg/L
Hoang P.pr. LC50 (<1d) pH6
BLM Hoang P.pr. LC50 (<1d) pH6
Hoang P.pr. LC50 (<1d) pH9
BLM Hoang P.pr. LC50 (<1d) pH9
Hoang P.pr. LC50 (28d) pH9
BLM Hoang P.pr. LC50 (28d) pH9
Hoang P.pr. LC50 (28d) pH7
BLM Hoang P.pr. LC50 (28d) pH7


A
BLM Predicted Ni Acute ECx (mg/L)
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-2}
10^{-1}
10^0
10^1
10^2
Measured Ni Acute ECx (mg/L)
D.magna
A.affinis (H)
B.coregoni (S)
C.lilljeborgi (H)
C.ovalis (H)
C.pulchella (H)
C.quadrangula (H)
C.quadrangula (S)
D.longispina (H)
D.magna
P.truncata (S)
S.serrulatus (S)
S.vetulus (H)
H.azteca
P.promelas
C.dubia
D.pulex
P.promelas
P.promelas
P.promelas
C.dubia
P.promelas
B
BLM Predicted Ni Chronic ECx (mg/L)
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
Measured Ni Chronic ECx (mg/L)
D.magna
A.affinis (H)
C.pulchella (H)
C.quadrangula (H)
C.quadrangula (S)
D.longispina (H)
O.mykiss
P.truncata (S)
S.serrulatus (S)
S.vetulus (H)
C.dubia

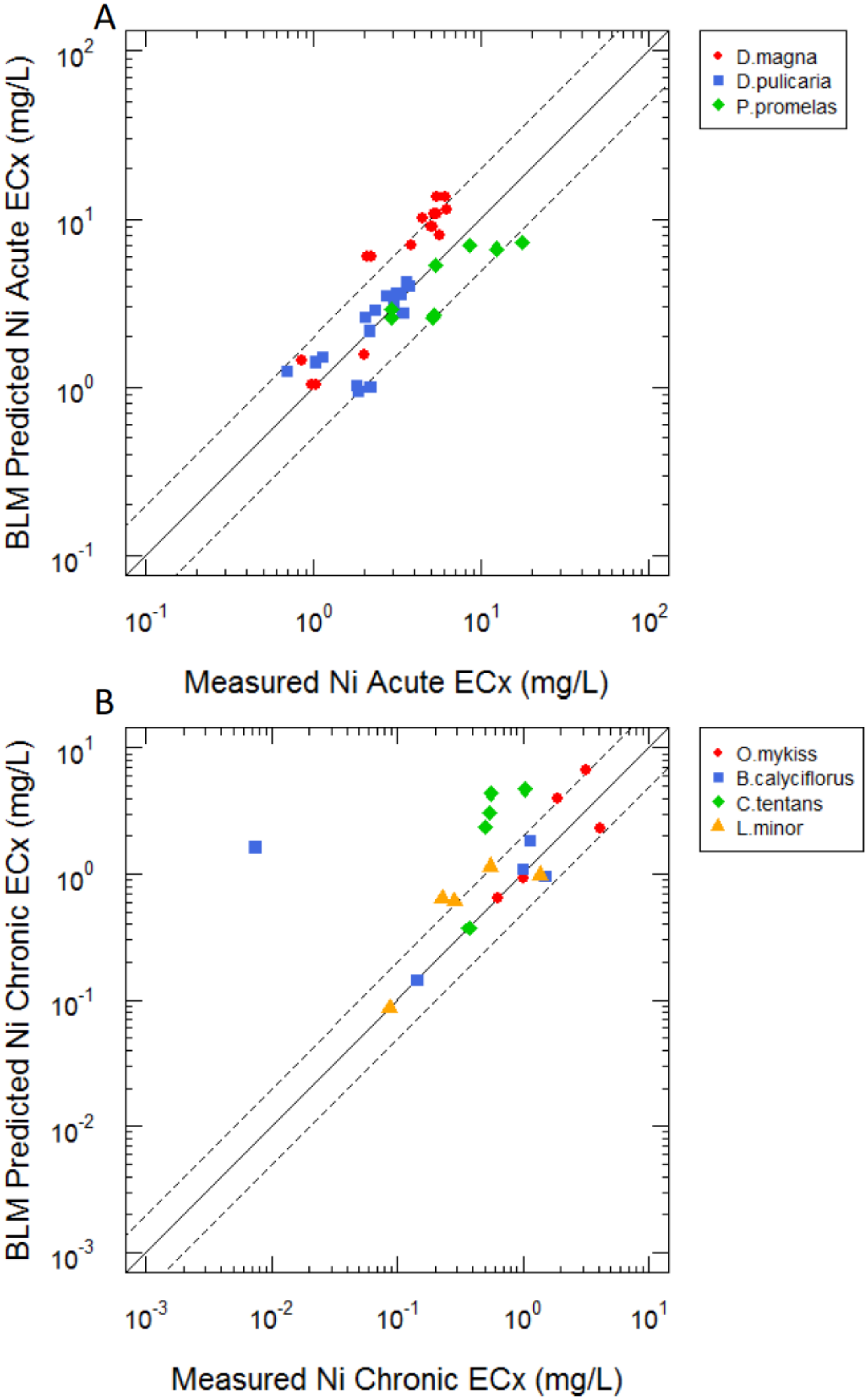

A
BLM Predicted Ni Acute ECx (mg/L)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-1}
10^{0}
10^{1}
10^{2}
Measured Ni Acute ECx (mg/L)
D.magna
D.pulicaria
P.promelas
B
BLM Predicted Ni Chronic ECx (mg/L)
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Measured Ni Chronic ECx (mg/L)
O.mykiss
B.calyciflorus
C.tentans
L.minor

Table 1 - Source Info

Species	Lifestage/Age	Exposure Duration	Reported Effect	Endpoint	# Obs	Study
Acute						
Alona affinis (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Bosmina coregoni (S)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Ceriodaphnia dubia	<1d	2d	LC50	survival	4	Keithly et al (2004)
Ceriodaphnia dubia	Not Reported	2d	LC50	survival	3	Schubauer-Berigan et al (1993)
Camptocercus lilljeborgi (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Chydorus ovalis (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Ceriodaphnia pulchella (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (S)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Daphnia longispina (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Daphnia magna	<1d	2d	EC50	immobilization	52	Deleebeeck et al (2008)
Daphnia magna	Not Reported	Not Rep.	LC50	survival	5	Chapman et al (manuscript)
Daphnia pulex	<1d	2d	LC50	immobilization	44	Kozlova et al (2009)
Daphnia pulicaria	Not Reported	2d	LC50	survival	16	Lind et al (1978)
Hyalella azteca	7-14d	2d	LC50	survival	20	Doig & Liber (2006)
Pimephales promelas	<1d	4d	LC50	survival	16	Hoang et al (2004 & unpublished)
Pimephales promelas	<1d	4d	LC50	survival	6	Pyle et al (2002)
Pimephales promelas	100d	4d	LC50	survival	4	Pickering (1974)
Pimephales promelas	28d ± 1	4d	LC50	survival	18	Hoang et al (2004 & unpublished)
Pimephales promelas	Not Reported	4d	LC50	survival	8	Lind et al (1978)
Pimephales promelas	Not Reported	4d	LC50	survival	3	Schubauer-Berigan et al (1993)
Pimephales promelas	subadult (1-6 g)	4d	LC50	survival	4	Meyer et al (1999)
Peracantha truncata (S)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Simocephalus serrulatus (S)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Simocephalus vetulus (H)	<2d	2d	EC50	immobilization	2	Deleebeeck et al (2007a)
Chronic						
Alona affinis (H)	<2d	16d	LC50	survival	2	Deleebeeck et al (2007a)
Brachionus calyciflorus	Not Reported	10d	EC10	population growth rate	5	Schlekat et al (2010)
Brachionus calyciflorus	Not Reported	10d	EC20	population growth rate	5	Schlekat et al (2010)
Ceriodaphnia dubia	<1d	7d	EC20	reproduction	3	Keithly et al (2004)
Ceriodaphnia dubia	<1d	7d	IC25	survival and reproduction	19	Parametrix (unpublished)
Ceriodaphnia dubia	<1d	7d	LC20	survival	3	Keithly et al (2004)
Ceriodaphnia pulchella (H)	<2d	17d	EC10	reproduction	2	Deleebeeck et al (2007a)
Ceriodaphnia pulchella (H)	<2d	17d	EC50	reproduction	2	Deleebeeck et al (2007a)
Ceriodaphnia pulchella (H)	<2d	17d	LC50	survival	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (H)	<2d	17d	EC10	reproduction	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (H)	<2d	17d	EC50	reproduction	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (H)	<2d	17d	LC50	survival	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (S)	<2d	17d	EC10	reproduction	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (S)	<2d	17d	EC50	reproduction	2	Deleebeeck et al (2007a)
Ceriodaphnia quadrangula (S)	<2d	17d	LC50	survival	2	Deleebeeck et al (2007a)
Chironomus tentans	Not Reported	10d	EC10	ash free dry weight	5	Schlekat et al (2010)
Chironomus tentans	Not Reported	10d	EC20	ash free dry weight	5	Schlekat et al (2010)
Daphnia longispina (H)	<2d	21d	EC10	reproduction	2	Deleebeeck et al (2007a)
Daphnia longispina (H)	<2d	21d	EC50	reproduction	2	Deleebeeck et al (2007a)
Daphnia longispina (H)	<2d	21d	LC50	survival	2	Deleebeeck et al (2007a)
Lemna minor	Not Reported	10d	EC10	growth rate	5	Schlekat et al (2010)
Lemna minor	Not Reported	10d	EC50	growth rate	5	Schlekat et al (2010)
Lymnea stagnalis	<1d	10d	EC10	wet weight	5	Schlekat et al (2010)
Lymnea stagnalis	<1d	10d	EC20	wet weight	5	Schlekat et al (2010)
Lymnea stagnalis	<1d	10d	EC50	wet weight	5	Schlekat et al (2010)
Onchorynchus mykiss	juvenile (28-35d)	17-26d	LC50	survival	20	Deleebeeck et al (2007b)
Peracantha truncata (S)	<2d	17d	EC10	reproduction	2	Deleebeeck et al (2007a)
Peracantha truncata (S)	<2d	17d	EC50	reproduction	2	Deleebeeck et al (2007a)
Peracantha truncata (S)	<2d	17d	LC50	survival	2	Deleebeeck et al (2007a)
Simocephalus serrulatus (S)	<2d	17d	EC10	reproduction	2	Deleebeeck et al (2007a)
Simocephalus serrulatus (S)	<2d	17d	EC50	reproduction	2	Deleebeeck et al (2007a)
Simocephalus serrulatus (S)	<2d	17d	LC50	survival	2	Deleebeeck et al (2007a)
Simocephalus vetulus (H)	<2d	21d	EC10	reproduction	2	Deleebeeck et al (2007a)
Simocephalus vetulus (H)	<2d	21d	EC50	reproduction	2	Deleebeeck et al (2007a)
Simocephalus vetulus (H)	<2d	21d	LC50	survival	2	Deleebeeck et al (2007a)

Table 2 - BL Parm

BL Species	**Old Log K**	**New Log K**
BL-Ni	4.000	4.000
BL-NiOH		-5.500
BL-Ca	4.000	4.250
BL-Mg		3.600
BL-Na		1.000
BL-H	6.700	4.700

Table 3 - Accum

Species	**Lifestage/Age**	**Exposure Duration**	**Reported Effect**	**Endpoint**	**SMEA (nmol/gw)**
			Acute		
A.affinis (H)	<2d	2d	EC50	immobilization	113.39
B.coregoni (S)	<2d	2d	EC50	immobilization	18.159
C.dubia	<1d	2d	LC50	survival	1.61385
C.dubia	Not Reported	2d	LC50	survival	0.73677
C.lilljeborgi (H)	<2d	2d	EC50	immobilization	22.669
C.ovalis (H)	<2d	2d	EC50	immobilization	108.1035
C.pulchella (H)	<2d	2d	EC50	immobilization	28.362
C.quadrangula (H)	<2d	2d	EC50	immobilization	14.086
C.quadrangula (S)	<2d	2d	EC50	immobilization	6.4518
D.longispina (H)	<2d	2d	EC50	immobilization	15.217
D.magna	<1d	2d	EC50	immobilization	46.4415
D.magna	Not Reported	Not Rep.	LC50	survival	27.416
D.pulex	<1d	2d	LC50	immobilization	52.555
D.pulicaria	Not Reported	2d	LC50	survival	14.333
H.azteca	7-14d	2d	LC50	survival	132.965
P.promelas	<1d	4d	LC50	survival	23.386
P.promelas	28d ± 1	4d	LC50	survival	172.59
P.promelas	Not Reported	4d	LC50	survival	54.351
P.promelas	subadult (1-6 g)	4d	LC50	survival	255.175
P.promelas	100d	4d	LC50	survival	134.89
P.truncata (S)	<2d	2d	EC50	immobilization	129.71
S.serrulatus (S)	<2d	2d	EC50	immobilization	54.111
S.vetulus (H)	<2d	2d	EC50	immobilization	25.7555
			Chronic		
A.affinis (H)	<2d	16d	LC50	survival	0.505365
B.calyciflorus	Not Reported	10d	EC10	population growth rate	4.1907
B.calyciflorus	Not Reported	10d	EC20	population growth rate	6.3275
C.dubia	<1d	7d	EC20	reproduction	0.024547
C.dubia	<1d	7d	IC25	survival and reproduction	0.0328955
C.dubia	<1d	7d	LC20	survival	0.048915
C.pulchella (H)	<2d	17d	EC10	reproduction	0.3944
C.pulchella (H)	<2d	17d	EC50	reproduction	0.605925
C.pulchella (H)	<2d	17d	LC50	survival	0.767295
C.quadrangula (H)	<2d	17d	EC10	reproduction	0.36252
C.quadrangula (H)	<2d	17d	EC50	reproduction	0.56079
C.quadrangula (H)	<2d	17d	LC50	survival	0.582045
C.quadrangula (S)	<2d	17d	EC10	reproduction	0.497135
C.quadrangula (S)	<2d	17d	EC50	reproduction	0.571685
C.quadrangula (S)	<2d	17d	LC50	survival	0.371325
C.tentans	Not Reported	10d	EC10	ash free dry weight	2.4169
C.tentans	Not Reported	10d	EC20	ash free dry weight	3.3679
D.longispina (H)	<2d	21d	EC10	reproduction	1.391635
D.longispina (H)	<2d	21d	EC50	reproduction	2.38145
D.longispina (H)	<2d	21d	LC50	survival	2.102
L.minor	Not Reported	10d	EC10	growth rate	0.14408
L.minor	Not Reported	10d	EC50	growth rate	1.5727
L.stagnalis	<1d	10d	EC10	wet weight	0.02264
L.stagnalis	<1d	10d	EC20	wet weight	0.044312
L.stagnalis	<1d	10d	EC50	wet weight	0.091653
O.mykiss	juvenile (28-35d)	17-26d	LC50	survival	22.2825
P.truncata (S)	<2d	17d	EC10	reproduction	0.611545
P.truncata (S)	<2d	17d	EC50	reproduction	1.363495
P.truncata (S)	<2d	17d	LC50	survival	1.1777
S.serrulatus (S)	<2d	17d	EC10	reproduction	1.07735
S.serrulatus (S)	<2d	17d	EC50	reproduction	1.28043
S.serrulatus (S)	<2d	17d	LC50	survival	1.19078
S.vetulus (H)	<2d	21d	EC10	reproduction	0.390875
S.vetulus (H)	<2d	21d	EC50	reproduction	0.48729
S.vetulus (H)	<2d	21d	LC50	survival	0.62758

Table 4 - Ion Ratios

Study,Species, lifestage	**Water Source**	**Ca:Mg (mol/mol)**	**Ca:Na (mol/mol)**	**Ca:K (mol/mol)**	**SO4:Cl (mol/mol)**	**Source of Ion Ratio**
Deleebeeck et al (2008), *Daphnia magna,* <1 day	Ankeveen	--	1.996	32.887	1.322	Deleebeeck 2008b & Gandhi 2011
	Bihain	--	0.267	3.205	0.137	Deleebeeck 2008b & Gandhi 2011
	Brisy	--	0.336	2.785	0.106	Deleebeeck 2008b
	Clywedog	--	0.308	4.207	0.254	Gandhi 2011
	Markermeer	--	0.392	6.078	0.354	Deleebeeck 2008b
	Mole	--	0.913	11.770	0.538	Gandhi 2011
	Regge	--	0.285	5.636	0.161	Deleebeeck 2008b
	Voyon	--	0.209	27.413	0.355	Deleebeeck 2008b
Lind et al (1978), *Daphnia pulicaria*	Colby L.	2.067	5.425	26.310	0.136	Chapra et al 2012 (all assumed similar to Lake Superior)
	Embarrass R.	2.067	5.425	26.310	0.136	
	Greenwood L.	2.067	5.425	26.310	0.136	
	L. Superior	2.067	5.425	26.310	0.136	
	S. Kawishiwi R.	2.067	5.425	26.310	0.136	
	St. Louis R.	2.067	5.425	26.310	0.136	
Lind et al (1978), *Pimephales promelas*	Colby L.	2.067	5.425	26.310	0.136	
	L. Superior	2.067	5.425	26.310	0.136	
	S. Kawishiwi R.	2.067	5.425	26.310	0.136	
	St. Louis R.	2.067	5.425	26.310	0.136	
Pyle et al (2002), *Pimephales promelas,* <1 day	Synthetic - Hardness=20	1.151	0.386	8.214	15.352	Pyle et al 2002 (dilution water info scaled to reported hardness)
	Synthetic - Hardness=55	1.262	1.184	25.190	45.153	
	Synthetic - Hardness=160	1.508	5.487	116.749	194.182	
	Synthetic - pH series	1.156	0.386	8.214	15.322	

Table 5 - Supplemental

Study,Species, lifestage	Exposure Type	Exposure Duration (days)	End point	Reported Effect	Water Source	Effect Conc	BLM Effect Conc	Temp	pH	DOC	Ca	Mg	Na	K	SO4	Cl	Alkalinity	Hardness	Comments
						mg/L				mg/L							mg/L as CaCO3		
Chapman et al (manuscript), *Daphnia magna*	Acute	Not Rep.	Survival	LC50	Synthetic	1.8019	1.4154	19.2	7.7	1.3	13.27	3.52	6.90	0.72	9.89	9.57	***44.16***	47.64	
						0.6280	1.4741	20.3	7.7	1.3	13.99	3.69	7.10	0.73	10.66	10.00	***43.20***	50.14	
						2.3595	2.3603	20.6	7.9	1.3	24.85	6.32	10.37	0.98	21.42	16.02	***83.25***	88.08	
						1.9193	2.4480	19.9	8.2	1.3	25.81	6.54	10.64	1.00	22.29	16.56	***84.12***	91.38	
						4.9713	4.4127	19.9	8.3	1.3	50.10	12.37	17.84	1.55	46.21	29.96	***166.00***	176.05	
Deleebeeck et al (2007a), *Alona affinis (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	5.5400	5.9867	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						2.9960	2.7856	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
	Chronic	16	Survival	LC50	Synthetic	0.0334	0.0265	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0099	0.0134	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007a), *Bosmina coregoni (S),* <2 day	Acute	2	Immobilization	EC50	Synthetic	0.5586	0.4161	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.1653	0.2490	20	7.18	***0.3***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
Deleebeeck et al (2007a), *Camptocercus lilljeborgi (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	1.0850	1.1009	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.5259	0.5190	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007a), *Ceriodaphnia pulchella (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	0.9810	1.3826	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.8417	0.6500	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
	Chronic	17	Reproduction	EC10	Synthetic	0.0276	0.0207	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0070	0.0105	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
				EC50	Synthetic	0.0312	0.0317	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0162	0.0160	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
			Survival	LC50	Synthetic	0.0360	0.0399	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0221	0.0201	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007a), *Ceriodaphnia quadrangula (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	0.5170	0.6820	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.4006	0.3234	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
	Chronic	17	Reproduction	EC10	Synthetic	0.0331	0.0191	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0025	0.0096	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
				EC50	Synthetic	0.0362	0.0293	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0113	0.0148	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
			Survival	LC50	Synthetic	0.0251	0.0304	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0180	0.0154	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007a), Ceriodaphnia quadrangula (S), <2 day	Acute	2	Immobilization	EC50	Synthetic	0.1406	0.1502	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0973	0.0918	20	7.18	***0.3***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
	Chronic	17	Reproduction	EC10	Synthetic	0.0217	0.0131	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0030	0.0087	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
				EC50	Synthetic	0.0234	0.0151	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0044	0.0100	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
			Survival	LC50	Synthetic	0.0139	0.0099	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0039	0.0066	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
Deleebeeck et al (2007a), *Chydorus ovalis (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	4.2560	5.6758	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						3.3350	2.6411	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007a), *Daphnia longispina (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	0.5106	0.7368	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.4555	0.3491	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
	Chronic	21	Reproduction	EC10	Synthetic	0.1130	0.0717	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0148	0.0360	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
				EC50	Synthetic	0.1250	0.1212	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0586	0.0605	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
			Survival	LC50	Synthetic	0.1180	0.1072	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	

						0.0483	0.0536	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007a), *Peracantha truncata (S),* <2 day	Acute	2	Immobilization	EC50	Synthetic	2.7260	3.2416	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						2.2000	1.8892	20	7.18	***0.3***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
	Chronic	17	Reproduction	EC10	Synthetic	0.0247	0.0161	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0049	0.0107	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
				EC50	Synthetic	0.0472	0.0353	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0153	0.0232	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
			Survival	LC50	Synthetic	0.0293	0.0306	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0210	0.0202	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
Deleebeeck et al (2007a), *Simocephalus serrulatus (S),* <2 day	Acute	2	Immobilization	EC50	Synthetic	1.4320	1.2580	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.6407	0.7396	20	7.18	***0.3***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
	Chronic	17	Reproduction	EC10	Synthetic	0.0453	0.0281	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0069	0.0185	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
				EC50	Synthetic	0.0542	0.0332	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0077	0.0218	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
			Survival	LC50	Synthetic	0.0473	0.0309	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
						0.0094	0.0204	20	7.18	***0.5***	1.75	0.46	2.61	0.17	3.98	1.65	12.58	6.25	
Deleebeeck et al (2007a), *Simocephalus vetulus (H),* <2 day	Acute	2	Immobilization	EC50	Synthetic	1.4850	1.2530	20	7.18	***0.3***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.4827	0.5899	20	7.18	***0.3***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
	Chronic	17	Reproduction	EC10	Synthetic	0.0233	0.0205	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0090	0.0104	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
				EC50	Synthetic	0.0289	0.0255	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0112	0.0129	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
			Survival	LC50	Synthetic	0.0388	0.0328	20	7.18	***0.5***	13.90	2.10	16.80	2.25	28.50	11.70	12.58	43.40	
						0.0135	0.0165	20	7.18	***0.5***	4.93	0.98	6.62	0.62	10.50	4.40	12.58	16.30	
Deleebeeck et al (2007b), *Onchorynchus mykiss,* juvenile (28-35 day)	Chronic	17-26	Survival	LC50	Synthetic	0.4960	0.5116	14.5	7.59	***0.3***	4.20	2.85	13.70	3.10	5.90	13.60	28.38	22.20	
						0.5190	0.5189	14.5	7.52	***0.3***	4.30	2.94	13.70	3.10	5.90	13.60	28.10	22.80	
						0.6620	1.2820	14.5	7.63	***0.3***	17.00	2.92	13.70	3.10	5.90	41.10	28.46	54.40	
						1.9500	2.6663	14.5	7.62	***0.3***	40.10	2.90	13.70	3.10	5.90	76.51	28.47	112.00	
						1.9900	6.8841	14.5	7.52	***0.3***	110.00	2.94	13.70	3.10	5.90	218.02	28.10	286.00	
						0.6730	0.4950	14.5	7.53	***0.3***	3.86	3.04	13.70	3.10	5.90	13.60	28.20	22.20	
						0.7960	0.6808	14.5	7.53	***0.3***	3.83	11.40	13.70	3.10	5.90	41.20	28.20	56.40	
						1.4800	0.9718	14.5	7.58	***0.3***	4.54	22.40	13.70	3.10	5.90	76.31	28.38	104.00	
						2.9100	1.4963	14.5	7.55	***0.3***	3.80	47.60	13.70	3.10	5.90	148.01	28.19	206.00	
						1.6200	2.0392	14.5	7.54	***0.3***	3.64	72.00	13.70	3.10	5.90	218.02	28.20	305.00	
						2.4400	0.5682	14.5	5.48	***0.3***	4.59	2.96	82.30	3.10	5.90	140.01	0.05	23.70	MOPS
						1.0200	0.5418	14.5	6.76	***0.3***	4.42	2.95	82.30	3.10	5.90	139.01	1.04	23.20	
						0.7810	0.5549	14.5	7.19	***0.3***	4.59	2.94	82.30	3.10	5.90	136.01	5.76	23.60	
						0.6140	0.5515	14.5	7.67	***0.3***	4.48	2.97	82.30	3.10	5.90	120.01	27.04	23.40	
						0.5580	0.5606	14.5	8.47	***0.3***	4.49	2.98	82.30	3.10	5.90	13.60	180.32	23.50	
					Ankeveen	3.2000	7.1699	14.5	7.55	18.4	83.00	13.70	15.30	2.65	81.11	29.10	36.55	263.00	
					Bihian	0.6400	0.7016	14.5	5.63	6.32	3.75	1.12	4.48	0.88	4.73	9.41	1.28	14.00	MOPS
					Brisy	1.0100	1.0093	14.5	7.39	3.83	7.53	4.54	6.80	2.29	8.65	16.20	23.63	37.50	
					Eppe	4.1400	2.4752	14.5	8.05	4.87	28.10	6.90	8.99	4.52	18.50	15.50	91.23	98.60	
					Markermeer	1.8900	4.2738	14.5	8.19	4.5	54.70	16.60	70.40	8.53	100.01	63.41	121.25	205.00	
Deleebeeck et al (2008), *Daphnia magna,* <1 day	Acute	2	Immobilization	EC50	Ankeveen	5.2500	9.8058	***20***	7.14	25.8	48.10	8.19	***13.82***	***0.40***	***112.21***	***31.33***	23.3	154.00	MOPS
						5.4400	9.8058	***20***	7.14	25.8	48.10	8.19	***13.82***	***0.40***	***112.21***	***31.33***	23.3	154.00	MOPS
						5.7200	7.3607	***20***	6.79	17.3	38.30	6.54	***11.01***	***0.32***	***82.68***	***23.08***	28.2	123.00	MOPS
					Bihain	2.2300	5.5150	***20***	6.23	6.62	34.50	1.47	***74.22***	***0.74***	***52.39***	***141.53***	0.390	92.10	MOPS
						2.1100	5.5150	***20***	6.21	6.62	34.50	1.47	***74.22***	***0.74***	***52.39***	***141.53***	0.390	92.10	MOPS

						0.8600	1.3245	***20***	6.15	5.37	3.68	1.07	***7.92***	***0.54***	***4.78***	***12.90***	8.36	13.60	MOPS
					Brisy	2.0100	1.4135	***20***	7.09	2.53	4.99	3.38	***8.51***	***1.95***	***5.83***	***20.21***	12.80	26.40	MOPS
					Clywedog	1.0400	0.9519	***20***	5.94	1.75	3.03	1.38	***5.63***	***0.53***	***8.29***	***12.06***	0.590	13.20	MOPS
						0.9800	0.9519	***20***	5.96	1.75	3.03	1.38	***5.63***	***0.53***	***8.29***	***12.05***	0.590	13.20	MOPS
					Markermeer	5.4900	11.9340	***20***	7.92	9.2	72.70	20.60	***106.27***	***5.45***	***154.05***	***160.47***	118	266.00	
						6.1300	11.9340	***20***	7.96	9.2	72.70	20.60	***106.27***	***5.45***	***154.07***	***160.48***	118	266.00	
						4.5200	8.8069	***20***	8.09	7.49	52.70	14.00	***77.04***	***3.71***	***93.64***	***97.54***	127	189.00	
					Mole	5.0100	8.0057	***20***	7.58	5.14	51.40	8.33	***32.28***	***1.14***	***72.08***	***49.48***	89.5	163.00	MOPS
						5.1300	8.0057	***20***	7.62	5.14	51.40	8.33	***32.28***	***1.14***	***72.09***	***49.48***	89.5	163.00	MOPS
					Regge	6.3000	9.7618	***20***	7.7	9.87	60.10	7.96	***121.03***	***2.27***	***67.58***	***154.46***	161	183.00	MOPS
					Voyon	3.8400	6.0583	***20***	8.02	4.17	37.10	7.13	***102.02***	***0.42***	***88.91***	***92.40***	122	122.00	
					Synthetic	1.8200	1.8157	***20***	6.5	***0.3***	8.86	5.86	2.31	3.05	24.00	23.80	2.28	***46.26***	
						2.0800	3.0622	***20***	6.63	***0.3***	18.60	5.69	2.16	3.05	24.00	41.50	2.55	***69.88***	
						2.5300	5.1384	***20***	6.71	***0.3***	34.80	5.49	2.13	3.05	24.00	77.00	2.71	***109.51***	
						3.4100	7.4593	***20***	6.77	***0.3***	52.90	5.25	2.11	3.05	24.00	112.00	2.82	***153.73***	
						3.5600	9.8123	***20***	6.89	***0.3***	71.10	5.18	2.08	3.05	24.00	149.00	3.03	***198.89***	
						4.4900	11.9927	***20***	6.89	***0.3***	87.80	5.57	1.79	3.05	24.00	185.00	3.03	***242.20***	
						5.5000	13.9566	***20***	6.86	***0.3***	103.00	5.40	1.79	3.05	24.00	220.00	2.98	***279.46***	
						3.4000	19.2918	***20***	6.9	***0.3***	144.00	5.32	2.05	3.05	24.00	291.00	3.04	***381.52***	
						2.2800	24.1152	***20***	6.92	***0.3***	181.00	5.30	2.02	3.05	24.00	362.00	3.07	***473.83***	
						2.2600	1.8600	***20***	6.62	***0.3***	9.12	6.05	1.79	3.05	24.00	23.80	2.53	***47.69***	
						2.6100	2.1223	***20***	6.58	***0.3***	9.03	11.70	1.79	3.05	24.00	41.50	2.45	***70.73***	
						2.9600	2.6527	***20***	6.6	***0.3***	8.92	22.90	1.79	3.05	24.00	77.00	2.49	***116.58***	
						3.2400	3.0888	***20***	6.48	***0.3***	8.23	33.70	1.79	3.05	24.00	112.00	2.24	***159.33***	
						3.5400	3.5451	***20***	6.59	***0.3***	8.70	41.80	1.79	3.05	24.00	149.00	2.47	***193.86***	
						3.7700	4.0459	***20***	6.79	***0.3***	8.55	52.50	1.79	3.05	24.00	185.00	2.86	***237.54***	
						4.0600	4.7081	***20***	6.82	***0.3***	8.56	66.10	1.79	3.05	24.00	220.00	2.91	***293.57***	
						3.6300	5.5556	***20***	6.85	***0.3***	8.10	84.60	1.79	3.05	24.00	291.00	2.96	***368.60***	
						3.5100	6.8166	***20***	6.75	***0.3***	7.87	111.00	1.79	3.05	24.00	362.00	2.79	***476.74***	
						3.1700	1.8097	***20***	6.8	***0.3***	8.80	5.82	1.79	3.05	24.00	23.80	2.88	***45.94***	
						3.4900	1.8180	***20***	6.8	***0.3***	8.74	5.84	23.00	3.05	24.00	56.50	2.88	***45.87***	
						3.4000	1.8434	***20***	6.8	***0.3***	8.84	5.77	46.00	3.05	24.00	92.00	2.88	***45.84***	
						3.2300	1.8651	***20***	6.8	***0.3***	8.74	5.85	92.00	3.05	24.00	163.00	2.88	***45.92***	
						3.5500	1.8306	***20***	6.8	***0.3***	8.35	5.55	138.00	3.05	24.00	234.00	2.88	***43.71***	
						3.1900	1.8494	***20***	6.8	***0.3***	8.27	5.59	184.00	3.05	24.00	306.00	2.88	***43.67***	
						3.3200	1.9622	***20***	6.8	***0.3***	9.10	5.11	230.00	3.05	24.00	377.00	2.88	***43.77***	
						3.6100	1.9169	***20***	6.8	***0.3***	8.51	5.17	276.00	3.05	24.00	448.00	2.88	***42.54***	
						3.6400	1.9365	***20***	6.8	***0.3***	8.36	5.46	322.00	3.05	24.00	519.00	2.88	***43.36***	
						2.5200	1.6716	***20***	5.95	***0.3***	7.04	6.32	92.00	3.05	24.00	145.00	0.110	***43.61***	
						2.6500	1.6605	***20***	6.28	***0.3***	7.03	6.26	92.00	3.05	24.00	145.00	0.570	***43.33***	
						2.8100	1.6692	***20***	6.74	***0.3***	7.10	6.26	92.00	3.05	24.00	143.00	2.77	***43.51***	
						2.6100	1.6768	***20***	7.24	***0.3***	7.09	6.33	92.00	3.05	24.00	156.00	14.20	***43.77***	
						3.0700	1.6566	***20***	7.53	***0.3***	6.98	6.31	92.00	3.05	24.00	116.00	46.90	***43.42***	
						3.2700	1.6328	***20***	8.13	***0.3***	6.89	6.29	92.00	3.05	24.00	23.00	198	***43.11***	
						2.3200	1.6557	***20***	5.72	***0.3***	7.08	6.35	41.70	3.05	24.00	78.40	0.740	***43.83***	MOPS
						2.5700	1.5812	***20***	6.07	***0.3***	6.63	6.29	47.70	3.05	24.00	72.50	1.34	***42.46***	MOPS
						2.7200	1.5981	***20***	6.63	***0.3***	6.75	6.33	60.40	3.05	24.00	58.90	2.55	***42.92***	MOPS
Doig & Liber (2006), *Hyalella azteca* , 7-14 days	Acute	2	Survival	LC50	Synthetic	13.8	12.598452	22.4	8.31	0.6	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						13.3	12.640711	22.4	8.31	0.8	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						12.94	12.911286	22.4	8.31	2.2	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	

						12.22	14.243617	22.4	8.31	9.2	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						14.79	12.604322	22.4	8.31	0.6	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						18.49	12.698817	22.4	8.31	1.1	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						16.2	13.607385	22.4	8.31	5.8	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						17.62	18.211264	22.4	8.31	30.4	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						15.43	12.622517	22.4	8.31	0.7	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						12.63	12.661841	22.4	8.31	0.9	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						12.6	12.954719	22.4	8.31	2.4	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						14.76	14.929738	22.4	8.31	12.7	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						13.32	12.645407	22.4	8.31	0.8	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						16.95	12.68121	22.4	8.31	1	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						14.08	13.091474	22.4	8.31	3.1	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						15.12	15.442715	22.4	8.31	15.4	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						12.07	12.594931	22.4	8.31	0.6	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						9.71	12.643059	22.4	8.31	0.9	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						12.04	12.905417	22.4	8.31	2.4	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
						12.66	14.514192	22.4	8.31	11.8	20.881	18.107	39.313	3.1357	121.61	2.8433	90	137.00	
Hoang et al (2004), *Pimephales promelas,* <1 day	Acute	4	Survival	LC50	Synthetic	0.7490	0.4917	25	6.07	2.16	2.45	2.38	4.68	0.57	20.65	3.45	3.56	20.00	
						0.7120	0.6959	25	6.17	6.28	2.52	2.38	19.52	0.81	37.07	8.52	4.08	20.00	
						0.7330	0.8590	25	6.05	9.77	2.82	2.29	30.66	0.71	50.98	12.68	4.87	20.00	
						2.2830	1.9122	25	8.81	2.02	16.25	14.32	174.81	2.39	99.29	4.58	375.86	101.00	
						2.9240	2.3345	25	8.8	6.24	16.40	14.58	190.34	2.71	120.89	9.81	374.85	100.00	
						3.5000	2.4753	25	8.79	8.63	15.50	13.66	190.27	2.39	135.20	16.63	370.83	102.00	
						0.3310	0.6514	25	7.41	2.44	3.86	1.23	5.45	1.71	4.64	5.69	17.56	14.00	
						0.2740	0.3848	25	7.34	0.5	2.07	1.53	5.43	0.56	11.47	0.58	15.67	12.00	
						1.4060	0.9848	25	7.36	2.3281	7.40	6.61	13.63	0.48	56.58	3.70	20.21	48.00	
						1.3620	1.2388	25	7.51	5.5227	7.48	6.58	24.23	0.50	73.78	8.73	22.67	48.00	
						1.1090	0.8793	25	7.4	0.5	7.78	6.82	7.19	0.40	45.36	0.84	20.19	47.00	
						1.3400	1.4605	25	7.59	8.2792	7.51	6.58	31.57	0.62	77.04	11.34	26.16	46.00	
						1.3160	1.0169	25	7.47	2.2657	7.82	6.81	12.88	0.57	53.24	2.78	20.16	47.00	
						1.2810	1.3047	25	7.21	6.9673	7.63	6.63	24.07	0.54	65.47	8.19	23.32	50.00	
Hoang et al (2004), *Pimephales promelas,* 28 day ± 1	Acute	4	Survival	LC50	Synthetic	10.8900	13.7277	25	8.61	0.5	15.22	14.11	174.71	2.22	97.27	1.87	386.72	101.00	
						14.0680	15.1187	25	8.61	6.49	15.50	13.61	194.21	2.34	125.64	15.25	382.71	101.00	
						13.3850	15.0818	25	8.62	3.45	16.34	14.35	175.31	2.23	112.12	4.92	381.71	100.00	
						14.3630	16.0966	25	8.6	9.32	16.02	14.12	183.77	2.30	123.78	9.30	386.71	101.00	
						5.4470	3.9988	25	6.06	0.5	3.23	2.66	1.29	0.49	19.70	0.47	4.32	19.00	
						8.6050	4.0676	25	6.18	2.39	2.74	2.44	5.81	0.53	33.20	3.01	4.29	21.00	
						9.9640	4.6263	25	6.16	5.72	2.66	2.39	16.98	0.57	48.45	7.44	4.30	20.00	
						9.6760	5.0286	25	6.04	8.13	2.66	2.35	28.83	0.64	62.43	11.48	5.41	20.00	
						5.4580	7.4006	25	7.22	0.6	7.68	6.70	6.89	0.56	57.72	0.76	19.26	49.00	
						7.4900	7.5520	25	7.32	2.1	7.39	6.65	12.40	0.51	65.28	3.38	19.21	49.00	
						8.0190	7.9594	25	7.34	4.7	7.17	6.53	23.94	0.60	81.98	8.21	22.24	48.00	
						7.7360	8.8180	25	7.41	8.1	7.49	6.50	34.83	0.61	100.42	12.66	27.25	48.00	
						7.9340	9.4384	25	7.34	9.75	8.24	6.76	6.98	0.43	54.47	0.43	28.30	47.00	
						6.3880	7.3043	25	7.2	0.6	7.36	6.39	35.03	0.62	89.91	11.68	19.27	47.00	
Hoang et al (this study), *Pimephales promelas,* <1 day	Acute	4	Survival	LC50	Synthetic	2.7420	1.7509	25	8.77	0.5	16.42	14.24	171.04	2.31	102.56	2.35	370.81	100.00	
						1.5110	1.6037	25	7.6	10.299	7.38	6.44	35.82	0.66	91.83	13.32	20.12	48.00	
Hoang et al (this study), *Pimephales promelas,* 28 day ± 1	Acute	4	Survival	LC50	Synthetic	12.1890	7.7164	25	7.24	0.5	8.24	6.79	6.96	0.54	60.55	0.56	20.26	51.00	
						11.3560	8.2006	25	7.32	2.03	8.56	6.71	11.02	0.59	67.43	2.61	22.25	51.00	
						12.7460	8.3884	25	7.33	3.31	8.38	6.73	17.52	0.59	79.89	6.05	23.25	51.00	

Table 5 - Supplemental

						12.0330	8.6414	25	7.38	6.12	7.86	6.54	26.57	0.59	95.01	9.65	25.25	51.00	
Keithly et al (2004), *Ceriodaphnia dubia,* <1 day	Acute	2	Survival	LC50	Synthetic	0.0810	0.0975	25	7.66	0.53	16.00	2.40	14.00	4E-06	51.00	1.10	24.40	50.00	
						0.1480	0.1722	25	7.7	0.5	31.00	8.70	15.00	4E-06	99.01	0.85	24.49	113.00	
						0.2610	0.2285	25	7.61	0.41	43.00	13.00	17.00	4E-06	180.01	2.10	22.70	161.00	
						0.4000	0.3483	25	7.8	0.43	68.00	20.00	16.00	4E-06	226.01	8.60	24.70	253.00	
	Chronic	7	Reproduction	EC20	Synthetic	0.0047	0.0037	25	7.7	1.3	31.00	8.70	15.00	4E-06	99.01	0.85	24.49	113.00	
						0.0040	0.0045	25	7.61	1.3	43.00	13.00	17.00	4E-06	180.01	2.10	22.70	161.00	
						0.0069	0.0069	25	7.8	1.3	68.00	20.00	16.00	4E-06	226.01	8.60	24.70	253.00	
			Survival	LC20	Synthetic	0.0072	0.0072	25	7.7	1.3	31.00	8.70	15.00	4E-06	99.01	0.85	24.49	113.00	
						0.0205	0.0088	25	7.61	1.3	43.00	13.00	17.00	4E-06	180.01	2.10	22.70	161.00	
						0.0129	0.0136	25	7.8	1.3	68.00	20.00	16.00	4E-06	226.01	8.60	24.70	253.00	
Kozlova et al (2009), *Daphnia pulex,* <1 day	Acute	2	Survival	LC50	Synthetic	0.4402	0.7496	20.5	7.85	0.15	0.80	3.40	13.10	1.06	15.37	0.96	28.11	***16.01***	
						0.9978	0.9271	20.5	7.85	0.15	2.00	3.40	13.10	1.06	18.25	0.96	28.11	***19.02***	
						1.2912	1.2228	20.5	7.85	0.15	4.01	3.40	13.10	1.06	23.05	0.96	28.11	***24.02***	
						0.9391	1.6929	20.5	7.85	0.15	7.21	3.40	13.10	1.06	30.74	0.96	28.11	***32.03***	
						2.6999	2.8106	20.5	7.85	0.15	14.83	3.40	13.10	1.06	48.99	0.96	28.11	***51.04***	
						5.3998	5.7459	20.5	7.85	0.15	34.87	3.40	13.10	1.06	97.02	0.96	28.11	***101.09***	
						5.2237	7.9734	20.5	7.85	0.15	50.10	3.40	13.10	1.06	133.53	0.96	28.11	***139.12***	
						7.7475	9.2659	20.5	7.85	0.15	58.91	3.40	13.10	1.06	154.66	0.96	28.11	***161.14***	
						1.8782	1.6340	20.5	7.85	0.15	6.81	3.40	13.10	0.20	29.78	0.18	28.11	***31.03***	
						2.4651	1.6340	20.5	7.85	0.15	6.81	3.40	13.10	4.69	29.78	4.25	28.11	***31.03***	
						1.9369	1.6384	20.5	7.85	0.15	6.81	3.40	13.10	9.77	29.78	8.86	28.11	***31.03***	
						1.9956	1.6384	20.5	7.85	0.15	6.81	3.40	13.10	17.59	29.78	15.95	28.11	***31.03***	
						1.9369	1.6439	20.5	7.85	0.15	6.81	3.40	13.10	30.50	29.78	27.65	28.11	***31.03***	
						1.6434	1.4525	20.5	7.85	0.15	6.81	0.24	13.10	1.06	17.29	0.96	28.11	***18.02***	
						1.7608	1.4946	20.5	7.85	0.15	6.81	0.97	13.10	1.06	20.17	0.96	28.11	***21.02***	
						2.3477	1.8429	20.5	7.85	0.15	6.81	7.05	13.10	1.06	44.19	0.96	28.11	***46.04***	
						1.7608	2.1171	20.5	7.85	0.15	6.81	11.91	13.10	1.06	63.40	0.96	28.11	***66.06***	
						3.1107	2.5672	20.5	7.85	0.15	6.81	19.93	13.10	1.06	95.10	0.96	28.11	***99.09***	
						2.9933	2.8504	20.5	7.85	0.15	6.81	25.03	13.10	1.06	115.27	0.96	28.11	***120.10***	
						3.6977	3.4031	20.5	7.85	0.15	6.81	35.00	13.10	1.06	154.66	0.96	28.11	***161.14***	
						1.5260	1.6225	20.5	6.3	0.15	6.81	3.40	4.60	1.06	29.78	7.09	0.85	***31.03***	
						1.4673	1.6292	20.5	6.71	0.15	6.81	3.40	11.50	1.06	29.78	17.73	0.83	***31.03***	
						1.5260	1.6393	20.5	6.81	0.15	6.81	3.40	22.99	1.06	29.78	35.45	0.83	***31.03***	
						1.4673	1.8049	20.5	7.85	1.53	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						1.6434	1.9667	20.5	7.85	2.84	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						3.0520	2.8266	20.5	7.85	9.8	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						5.2237	3.6633	20.5	7.85	16.5	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						5.0476	4.4744	20.5	7.85	22.9	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						7.0432	6.0865	20.5	7.85	35.3	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						1.2912	1.6468	20.5	5.6	0.15	6.81	3.40	0.46	1.06	29.78	0.96	1.11	***31.03***	
						1.5260	1.6294	20.5	7.6	0.15	6.81	3.40	4.60	1.06	29.78	0.96	10.07	***31.03***	
						1.4086	1.6374	20.5	7.9	0.15	6.81	3.40	20.00	1.06	29.78	0.96	42.17	***31.03***	
						1.3499	1.6417	20.5	8	0.15	6.81	3.40	22.99	1.06	29.78	0.96	52.13	***31.03***	
						1.5260	1.6527	20.5	5.6	0.15	6.81	3.40	13.10	1.06	29.78	0.96	30.97	***31.03***	MOPS
						1.4086	1.6258	20.5	6.3	0.15	6.81	3.40	13.10	1.06	29.78	0.96	29.78	***31.03***	MOPS
						2.1716	1.6288	20.5	7	0.15	6.81	3.40	13.10	1.06	29.78	0.96	28.61	***31.03***	MOPS
						3.1694	1.6327	20.5	7.5	0.15	6.81	3.40	13.10	1.06	29.78	0.96	28.22	***31.03***	MOPS
						3.9911	1.6364	20.5	8	0.15	6.81	3.40	13.10	1.06	29.78	0.96	28.08	***31.03***	MOPS
						3.9324	1.6516	20.5	8.3	0.15	6.81	3.40	13.10	1.06	29.78	0.96	28.05	***31.03***	MOPS

						0.9978	1.6774	20.5	7.85	0.5	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						2.9347	2.8514	20.5	7.85	10	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						2.8173	4.0799	20.5	7.85	19.8	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						2.4651	5.2990	20.5	7.85	29.3	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
						3.2281	6.8483	20.5	7.85	41	6.81	3.40	13.10	1.06	29.78	0.96	28.11	***31.03***	
Lind et al (1978), *Daphnia pulicaria*	Acute	2	Survival	LC50	Colby L.	2.0420	2.3242	18	7.41	18	***24.02***	***7.05***	***2.54***	***0.89***	***16.55***	***44.87***	14.00	89.00	
						2.7170	3.1259	18	7.09	34	***24.02***	***7.05***	***2.54***	***0.89***	***16.30***	***44.21***	14.00	89.00	
					Embarrass R.	3.1560	3.2538	18	7.43	27	***30.77***	***9.03***	***3.25***	***1.14***	***15.68***	***42.53***	43.00	114.00	
						3.6070	3.7662	18	7.47	33	***32.38***	***9.50***	***3.42***	***1.20***	***18.38***	***49.84***	37.00	120.00	
					Greenwood L.	2.1710	1.9282	18	5.88	39	***6.75***	***1.98***	***0.71***	***0.25***	***3.67***	***9.94***	2.50	25.00	
					L. Superior	2.1820	0.8644	18	8.07	2.6	***12.95***	***3.80***	***1.37***	***0.48***	***1.86***	***5.03***	42.00	48.00	
						1.8130	0.8823	18	8.1	2.8	***12.95***	***3.80***	***1.37***	***0.48***	***1.45***	***3.94***	44.00	48.00	
						1.8360	0.8248	18	8.04	2.7	***11.87***	***3.48***	***1.26***	***0.44***	***0.96***	***2.61***	42.00	44.00	
					S. Kawishiwi R.	0.6970	1.1045	18	6.77	13	***7.83***	***2.30***	***0.83***	***0.29***	***0.01***	***0.03***	26.00	29.00	
						1.1400	1.3603	18	7.23	15	***7.56***	***2.22***	***0.80***	***0.28***	***1.13***	***3.07***	22.00	28.00	
						1.0340	1.2715	18	7.36	13	***7.56***	***2.22***	***0.80***	***0.28***	***1.72***	***4.65***	20.00	28.00	
					St. Louis R.	3.3160	3.2236	18	7.25	34	***23.21***	***6.81***	***2.45***	***0.86***	***13.96***	***37.85***	22.00	86.00	
						3.0140	2.8880	18	7.01	32	***22.67***	***6.65***	***2.40***	***0.84***	***14.37***	***38.97***	17.00	84.00	
						2.3250	2.5889	18	7.09	28	***19.97***	***5.86***	***2.11***	***0.74***	***12.28***	***33.29***	17.00	74.00	
						3.4140	2.4800	18	6.94	28	***19.70***	***5.78***	***2.08***	***0.73***	***11.57***	***31.37***	18.00	73.00	
						3.7570	3.6407	18	7.55	34	***26.99***	***7.92***	***2.85***	***1.00***	***17.74***	***48.10***	20.00	100.00	
Lind et al (1978), *Pimephales promelas*	Acute	4	Survival	LC50	Colby L.	5.3830	6.0383	25	7.16	15	***23.21***	***6.81***	***2.45***	***0.86***	***14.78***	***40.07***	18.00	86.00	
					L. Superior	5.2090	3.0429	25	8.05	4.2	***12.14***	***3.56***	***1.28***	***0.45***	***0.98***	***2.65***	43.00	45.00	
						5.1630	2.9227	25	8.01	3.7	***11.87***	***3.48***	***1.26***	***0.44***	***0.95***	***2.57***	42.00	44.00	
					S. Kawishiwi R.	2.9160	2.9162	25	6.5	12	***7.83***	***2.30***	***0.83***	***0.29***	***0.01***	***0.03***	20.00	29.00	
						2.9230	3.2519	25	7	14	***7.56***	***2.22***	***0.80***	***0.28***	***1.01***	***2.73***	21.00	28.00	
					St. Louis R.	12.3560	7.4029	25	6.99	32	***20.78***	***6.10***	***2.20***	***0.77***	***12.30***	***33.35***	19.00	77.00	
						17.6780	8.1472	25	7.09	33	***24.02***	***7.05***	***2.54***	***0.89***	***14.87***	***40.33***	20.00	89.00	
						8.6170	7.8690	25	7.04	30	***24.56***	***7.21***	***2.60***	***0.91***	***15.47***	***41.96***	19.00	91.00	
Meyer et al (1999), *Pimephales promelas,* subadult (1-6 g)	Acute	4	Survival	LC50	Synthetic	8.8040	8.2822	20	7.47	0.3	5.37	2.45	0.86	2.40	9.70	0.69	25.19	***23.52***	
						21.3057	26.3696	20	7.27	0.3	25.37	2.45	1.00	0.58	19.50	30.74	23.91	***73.46***	
						56.1696	55.9661	20	7.2	0.3	57.71	2.84	0.97	0.43	43.23	103.88	24.80	***155.83***	
						89.8009	90.2757	20	7.34	0.3	95.39	2.72	0.85	1.11	103.75	165.21	23.40	***249.41***	
Parametrix (unpublished), *Ceriodaphnia dubia,* <1 day	Chronic	7	Survival & Reproduction	IC25	Synthetic	0.0019	0.0041	25	8.5	***0.3***	29.20	9.71	44.40	2.78	88.20	1.97	96.00	96.00	
						0.0022	0.0054	25	8.4	***0.3***	42.60	14.30	41.20	4.37	145.00	3.52	95.00	154.00	
						0.0034	0.0098	25	8.4	***0.3***	86.70	28.80	46.00	9.49	269.00	7.54	95.00	292.00	
						0.0016	0.0051	25	8.7	***0.3***	34.70	18.70	93.30	6.05	176.00	4.53	194.00	194.00	
						0.0060	0.0066	25	8.6	***0.3***	48.60	31.50	99.30	10.50	281.00	7.64	196.00	310.00	
						0.0094	0.0136	25	8.4	***0.3***	122.00	54.90	102.00	16.90	524.00	15.60	197.00	586.00	
						0.0070	0.0024	25	8	***0.3***	17.50	3.72	8.18	1.00	36.70	0.62	26.00	42.00	
						0.0065	0.0036	25	8.1	***0.3***	27.50	7.36	9.03	2.09	66.10	1.51	25.00	76.00	
						0.0314	0.0240	25	7.8	***0.3***	237.00	77.60	10.90	27.40	790.00	22.80	24.00	848.00	
					Desjardins Cnl	0.0499	0.0233	25	8.3	5	64.50	17.40	90.50	11.30	62.80	157.00	81.00	232.00	
					Desjardins Cnl (filtered)	0.0361	0.0290	25	8.3	6.7	65.30	17.40	95.90	11.40	70.80	172.00	84.00	230.00	
					Synthetic	0.0428	0.0301	25	8.4	6	72.70	19.30	95.10	11.70	70.70	172.00	91.00	262.00	
						0.0149	0.0073	25	8.4	***0.3***	60.60	22.60	40.10	15.90	245.00	15.10	86.00	246.00	
					Grand R.	0.0193	0.0375	25	8.6	7.3	62.60	21.30	68.00	4.87	50.20	106.00	165.00	234.00	
					Grand R. (filtered)	0.0153	0.0380	25	8.6	7.5	61.60	21.20	68.80	4.81	49.20	107.00	166.00	236.00	
					Synthetic	0.0170	0.0354	25	8.6	8.4	51.10	26.60	95.80	5.50	160.00	247.00	165.00	236.00	
						0.0047	0.0064	25	8.5	***0.3***	50.60	21.40	78.50	7.41	224.00	6.26	158.00	228.00	

Table 5 - Supplemental

					Poudre R.	0.0389	0.0279	25	8.3	6.8	53.40	13.30	46.80	7.56	86.80	29.10	102.00	170.00	
					Poudre R. (filtered)	0.0315	0.0291	25	8.4	6.6	52.60	13.40	46.10	7.45	86.20	28.90	102.00	184.00	
Pickering (1974), *Pimephales promelas,* 100 day	Acute	4	Survival	LC50	Synthetic	42.3766	45.6555	25	8.2	1	96.99	28.68	105.06	8.37	325.65	58.85	***300.13***	360.31	
						44.4896	45.6555	25	8.2	1	96.99	28.68	105.06	8.37	325.65	61.33	***300.13***	360.31	
						5.1826	4.4641	25	7.5	0.5	5.37	1.59	105.06	8.37	325.65	13.90	***18.09***	19.98	
						4.5781	4.4641	25	7.5	0.5	5.37	1.59	105.06	8.37	325.65	13.15	***18.09***	19.98	
Pyle et al (2002), *Pimephales promelas,* <1 day	Acute	4	Survival	LC50	Synthetic	0.4500	0.5240	25	6.9	***0.3***	***4.28***	***2.26***	***6.37***	***0.51***	***19.20***	***0.46***	13.60	20.00	
						0.5000	1.1267	25	7	***0.3***	***12.29***	***5.91***	***5.95***	***0.48***	***52.79***	***0.43***	13.60	55.00	
						2.2700	3.0232	25	7.6	***0.3***	***38.52***	***15.49***	***4.03***	***0.32***	***153.57***	***0.29***	20.40	160.00	
						0.6900	1.0346	25	5.5	***0.3***	***10.73***	***5.63***	***15.95***	***1.27***	***47.99***	***1.16***	31.50	50.00	
						0.5400	1.0235	25	7	***0.3***	***10.73***	***5.63***	***15.95***	***1.27***	***47.99***	***1.16***	31.50	50.00	
						2.2100	1.0631	25	8.5	***0.3***	***10.73***	***5.63***	***15.95***	***1.27***	***47.99***	***1.16***	31.50	50.00	
Schlekat et al (2010), *Brachionus calyciflorus,* <1 day	Chronic	10	Population Growth Rate	EC10	Calapooia, OR	0.6626	0.6632	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.9452	1.2344	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.0047	1.1119	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.1039	0.0974	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	1.3793	0.6780	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
				EC20	Calapooia, OR	0.9904	0.9919	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	1.1411	1.7582	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.0074	1.6365	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.1439	0.1424	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	1.4976	0.9590	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
Schlekat et al (2010), *Chironomus tentans,* <1 day	Chronic	10	Ash-free Dry Weight	EC10	Calapooia, OR	0.4043	0.3894	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.7822	0.7802	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.4054	0.6645	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.3057	0.0584	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.2511	0.4296	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
				EC20	Calapooia, OR	0.5443	0.5365	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	1.0288	1.0269	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.5510	0.9064	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.3723	0.0796	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.5017	0.5652	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
Schlekat et al (2010), *Lemna minor,* <1 day	Chronic	10	Growth Rate	EC10	Calapooia, OR	0.0075	0.0270	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.0739	0.0786	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.4353	0.0440	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.0082	0.0038	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.0360	0.0360	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
				EC50	Calapooia, OR	0.2288	0.2579	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.5509	0.5510	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	1.3770	0.4439	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.0870	0.0390	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.2844	0.3011	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
Schlekat et al (2010), *Lymnea stagnalis,* <1 day	Chronic	10	Wet Weight	EC10	Calapooia, OR	0.0011	0.0045	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.0195	0.0141	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.0070	0.0070	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.0014	0.0006	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.0013	0.0059	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
				EC20	Calapooia, OR	0.0016	0.0087	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.0268	0.0268	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.0213	0.0136	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2

Table 5 - Supplemental

					S. Santium R., OR	0.0063	0.0012	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.0019	0.0115	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
				EC50	Calapooia, OR	0.0062	0.0175	25	8	0.69	46.00	21.00	68.00	1.90	1.20	73.00	200.00	212.00	
					S. Platte R., CO	0.0398	0.0526	25	7.8	7.1	71.00	17.00	120.00	14.00	150.00	120.00	160.00	256.00	
					S. Platte R., CO (pH adj)	0.0779	0.0281	25	6.9	7	72.00	17.00	120.00	47.00	150.00	150.00	16.00	256.00	CO2
					S. Santium R., OR	0.0148	0.0024	25	7.1	0.94	3.40	0.81	2.40	0.25	0.51	0.98	24.00	16.00	
					Zollner Cr., OR	0.0233	0.0233	25	7.3	7.1	32.00	11.00	3.90	1.00	3.00	65.00	28.00	136.00	
Schubauer-Berigan et al (1993), *Ceriodaphnia dubia*	Acute	2	Survival	LC50	Synthetic	0.2001	0.1341	25	6.51	1	47.69	41.56	105.06	8.37	325.65	7.87	***245.63***	290.25	
						0.1403	0.1403	25	7.28	1	47.69	41.56	105.06	8.37	325.65	7.80	***237.41***	290.25	
						0.0130	0.1609	25	8.73	1	47.69	41.56	105.06	8.37	325.65	7.62	***235.18***	290.25	
Schubauer-Berigan et al (1993), *Pimephales promelas*	Acute	4	Survival	LC50	Synthetic	4.0029	10.2648	25	6.71	1	47.69	41.56	105.06	8.37	325.65	12.44	***242.69***	290.25	
						3.3983	10.2531	25	7.43	1	47.69	41.56	105.06	8.37	325.65	11.73	***236.81***	290.25	
						3.0990	10.2754	25	8.58	1	47.69	41.56	105.06	8.37	325.65	11.38	***235.13***	290.25	

Exhibit 30

Table 2

Exhibit 30

	River Upstream Total Nickel	Plant Effluent Total Nickel	River Downstream Total Nickel
Month	mg/L	mg/L	mg/L
March-07	<0.005	0.016	<0.005
April-07	<0.005	0.016	<0.005
May-07	<0.005	0.019	<0.005
June-07	<0.005	0.022	0.009
July-07	<0.005	0.025	0.011
August-07	<0.005	0.028	0.026
September-07	<0.005	0.027	0.025
October-07	<0.005	0.023	0.02
November-07	<0.005	0.022	0.019
December-07	<0.005	0.021	<0.007
January-08	<0.005	0.022	<0.005
February-08	<0.010	0.027	<0.010
March-08	<0.005	0.028	<0.005
April-08	<0.005	0.028	<0.006
May-08	<0.005	0.023	<0.005
June-08	<0.005	0.034	<0.005
July-08	<0.005	0.028	<0.005
August-08	<0.005	0.039	0.024
September-08	<0.005	0.027	<0.007
October-08	<0.005	0.028	<0.005
November-08	<0.005	0.038	0.007
December-08	<0.005	0.033	<0.006
January-09	0.001	0.031	0.005
February-09	0.002	0.024	0.005
March-09	0.002	0.019	0.003
April-09	0.002	0.016	0.002
May-09	0.003	0.029	0.004
June-09	0.003	0.023	0.007
July-09	0.002	0.024	0.004
August-09	0.002	0.023	0.01
September-09	0.002	0.027	0.014
October-09	0.001	0.024	0.005
November-09	0.003	0.019	0.003
December-09	<0.001	0.018	<0.002
January-10	0.002	0.018	0.003
February-10	0.002	0.025	0.002
March-10	0.001	0.022	0.002
April-10	<0.001	0.019	0.003
May-10	0.001	0.017	0.002
June-10	0.002	0.022	0.003
July-10	<0.002	0.045	0.006
August-10	0.002	0.044	0.031
September-10	<0.002	0.024	0.013
October-10	0.002	0.021	0.015
November-10	0.001	0.021	0.018
December-10	<0.002	0.019	0.002

January-11	<0.001	0.019	0.008
February-11	<0.001	0.016	0.006
March-11	<0.002	0.014	<0.002
April-11	<0.001	0.014	0.002
May-11	<0.002	0.012	0.002
June-11	<0.002	0.015	0.003
July-11	<0.001	0.017	0.012
August-11	<0.001	0.021	0.019
September-11	<0.001	0.022	0.021
October-11	<0.001	0.024	0.022
November-11	<0.002	0.026	0.028
December-11	<0.001	0.022	0.014
January-12	<0.001	0.023	0.015
February-12	<0.001	0.024	0.008
March-12	<0.001	0.028	0.014
April-12	<0.001	0.031	0.020
May-12	0.002	0.030	0.008
June-12	0.002	0.031	0.019
July-12	0.002	0.032	0.023
August-12	0.002	0.035	0.027
September-12	0.002	0.029	0.020
October-12	0.002	0.031	0.018
November-12	0.002	0.042	0.031
December-12	0.002	0.036	0.025
January-13	<0.002	0.035	0.007
February-13	<0.002	0.030	0.003
March-13	<0.002	0.032	<0.002
April-13	<0.004	0.022	<0.004
May-13	<0.002	0.019	<0.002
June-13	<0.002	0.022	<0.002
July-13	<0.002	0.031	0.005
August-13	<0.002	0.004	0.029
September-13	<0.002	0.047	0.033
October-13	<0.002	0.084	0.059
November-13	<0.002	0.061	0.038
December-13	<0.002	0.035	0.029
January-14	<0.002	0.033	0.022
February-14	<0.002	0.021	0.028
March-14	<0.002	0.022	0.006
April-14	<0.002	0.015	<0.003
May-14	<0.002	0.014	<0.004
June-14	<0.002	0.023	0.003
July-14	<0.002	0.024	0.003
August-14	<0.002	0.025	0.007
September-14	<0.002	0.020	0.005
October-14	<0.002	0.017	<0.002
November-14	<0.002	0.022	0.003
December-14	<0.002	0.019	<0.002
January-15	<0.002	0.019	<0.002
February-15	<0.002	0.017	<0.002
March-15	<0.002	0.015	<0.002

April-15	<0.002	0.016	<0.002
May-15	<0.002	0.015	<0.002
June-15	<0.002	0.018	<0.002
July-15	<0.002	0.019	<0.002
August-15	<0.002	0.025	0.009
September-15	<0.002	0.025	0.022
October-15	<0.002	0.032	0.016
November-15	<0.002	0.056	0.049
December-15	<0.002	0.019	<0.002
January-16	0.003	0.017	<0.002
February-16	<0.002	0.022	<0.002
March-16	0.006	0.021	0.004
April-16	0.006	0.020	0.005
May-16	0.003	0.020	0.004
June-16	0.003	0.025	0.003
July-16	0.002	0.023	0.004
August-16	<0.002	0.020	0.004
September-16	<0.002	0.021	<0.002
October-16	<0.002	0.024	0.009
November-16	<0.002	0.020	0.006
December-16	<0.002	0.023	<0.002
January-17	<0.002	0.019	<0.002
February-17	<0.002	0.018	<0.002
March-17	<0.002	0.016	0.002
April-17	<0.002	0.017	0.003
May-17	0.003	0.013	0.003
June-17	<0.002	0.023	0.003
July-17	<0.002	0.020	0.012
August-17	<0.002	0.019	0.017
September-17	<0.002	0.019	0.025
October-17	<0.002	0.020	0.016

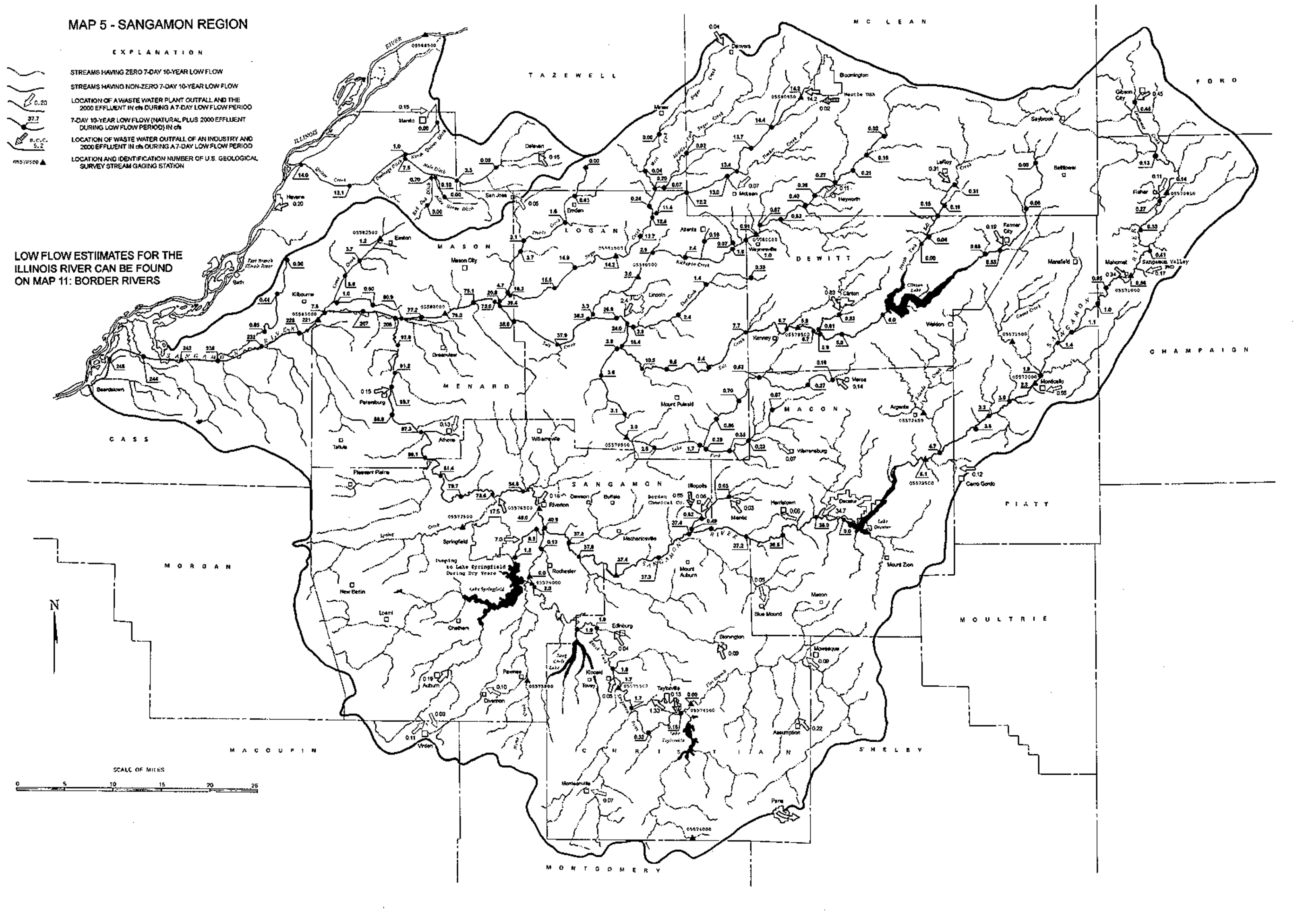
Exhibit 31

Exhibit 31

MAP 5 SANGAMON REGION

SANGAMON RIVER WITH SALT CREEK AND OTHER TRIBUTARIES

APRIL 2002 REVISION



Illinois State Water Survey

Exhibit 32

Exhibit 32

Ecological condition of a stretch of the Sangamon River receiving effluent from the Sanitary District of Decatur: Focusing on water chemistry, qualitative habitat assessment, and the mussel, macroinvertebrate, and fish assemblages

Robert E. Colombo, Ph.D.
Department of Biological Sciences
Eastern Illinois University
PHONE: (217) 581-3011
FAX: (217) 581-7141
recolombo@eiu.edu

Jeffrey R. Laursen, Ph.D.
Department of Biological Sciences
Eastern Illinois University
PHONE: (217) 581-3914
FAX: (217) 581-7141
jrlaursen@eiu.edu

Charles L. Pederson, Ph.D.
Department of Biological Sciences
Eastern Illinois University
PHONE: (217) 581-6239
FAX: (217) 581-7141
clpederson@eiu.edu

Research Assistants:

Bethany Hoster, bhoster@eiu.edu
Samuel Gradle, sjgradle@eiu.edu

Submitted to:
Sanitary District of Decatur
501 Dipper Lane
Decatur, IL 62522

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TABLE OF CONTENTS

Table of Contents

EXECUTIVE SUMMARY

We sampled two treatment reaches of the Sangamon River for water quality and aquatic macroinvertebrate, freshwater mussel, and fish assemblages. The two treatment reaches were 1) upstream of the Decatur Sanitary District main discharge (downstream of the Lake Decatur Dam), and 2) downstream of the main discharge. We sampled six sites monthly for water quality; one site located in the upstream reach, and five sites located downstream of the SDD. Seven sites were sampled during fall 2016 for macroinvertebrate assemblages; three sites located in the upstream reach and four located in the downstream reach. Six sites were sampled during fall 2016 for fish assemblages, including three sites located in the upstream reach and three sites located in the downstream reach.

Water quality in the upstream and downstream reaches differed during periods when discharge, measured at the Route 48 Bridge, was below 200 cfs. Macroinvertebrate indices such as estimated abundance, percent EPT, richness, EPT richness, and MBI showed differences between the two reaches, all being higher (except for MBI which was lower) downstream of the SDD main effluent outfall. A single season indicted that there were differences in macroinvertebrate assemblages between microhabitat types, and when comparing single microhabitats between reaches. Ongoing studies are evaluating specific microhabitats to try to discern critical habitat between the reaches. A total of twenty-one fish species was sampled using pulsed DC electrofishing from the two treatment reaches of the Sangamon River. Catch per unit effort was highest in the upstream reach (Site 5) and lowest in the downstream reach (Site 9). Species from the family Catostomidae comprised more than 56% of the total sample.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Rivers and streams are impounded for a variety of reasons, including residential, commercial, and agricultural water supply, flood and debris control, and hydropower production (Kondolf 1997). However, impoundments may impact the aquatic systems downstream and their surrounding terrestrial habitats. Impoundments can affect riverine systems by altering the flow regime, changing the sediment and nutrient loads, and modifying energy flow (Lignon *et al.* 1995). In addition, impoundments may lead to diminished water quality and availability, closures of fisheries, extirpation of species, and groundwater depletion for surrounding areas (Abramovitz 1996, Collier *et al.* 1996, Naiman *et al.* 1995). As a result, downstream reaches may no longer be able to support native species, which is reflected by reduced integrity of biotic communities (Naiman *et al.* 1995, NRC 1992).

A natural flow regime is critical for sustaining ecosystem integrity and native biodiversity in rivers (Poff *et al.* 1997). Depending on the use of the dam, it may have varying effects on downstream aquatic habitats. Impoundments used for urban water supplies lead to a reduction in flow rates downstream of the dam throughout the entire year (Finlayson *et al.* 1994), as well as increase daily and seasonal variability in flow regime (Finlayson *et al.* 1994, McMahon & Finlayson 2003). In addition, abiotic variables including temperature, dissolved oxygen, turbidity, pH, conductivity, and solids concentrations are altered in the downstream river system (Finlayson *et al.* 1994).

Along with stream and river impoundments, point source and non-point source pollution can have profound and lasting effects on the ecological integrity of the system. Non-point sources of pollution include agriculture, livestock grazing, and urbanization. Point source pollution includes sanitary discharge and industrial waste. In order to reduce point source pollution, the Water Quality Act of 1972 encouraged wastewater treatment plants to upgrade their systems. As a result, many communities were forced to build advanced tertiary water treatment facilities (Karr *et al.* 1985). Updated facilities still release high concentrations of nutrients into surrounding rivers. Carpenter and Waite (2000) documented that concentrations of phosphorus were highest in streams draining agricultural basins and at sites influenced by wastewater discharges. Twichell et al. (2002) reported that sewage effluent inputs had elevated nitrate levels. The enhanced nutrient discharge can be expected to increase productivity within a river because primary productivity and detrital processing usually are limited by low ambient stream nutrient concentrations (Stockner and Shortreed 1978, Elwood et al. 1981, Winterbourn 1990).

Fluctuating water levels, either dictated by outflow over a dam or naturally occurring drought, can also severely affect aquatic ecosystems. Low flow can alter the lotic systems in ways harmful to biota, including loss of habitat, food resources, and stream connectivity (Lake 2003). The overall effect drought has on aquatic communities varies, and often depends on the availability of refugia and the life history of the organisms (Humpheries and Baldwin 2003, Lake 2003). Macroinvertebrates, especially sensitive taxa such as stoneflies and caddisflies, can be temporarily decimated by drought conditions (Boulton 2003). In flashy rivers, immobile taxa such as mussels are confined to small refugia remaining at low flow even when water levels increase. Human disturbances such as impoundments can be exacerbated by low flow conditions, decreasing the amount of dilution for pollution sources in lotic systems. This can lower the resilience of the aquatic ecosystem, potentially worsening their effects (Bond *et al.* 2008).

The Sangamon River

The Sangamon River, the largest tributary to the Illinois River, flows for approximately 200 km in central Illinois, and its 14,000 km^2 watershed extends to 18 counties. Streams converging with the Sangamon River run through glacial and alluvial deposits, creating a low gradient stream with sand and gravel substrates. The Sangamon basin has experienced multiple point and non-point source impacts throughout the years. The Sangamon River watershed is dominated by agriculture and has the highest percentage of its land in crops of all major watersheds in the state (IDNR 2001). Major cities along the river include Bloomington, Decatur, and Springfield, and are home to more than 500,000 people. The Sanitary District of Decatur (SDD) serves more than 100,000 people and 24 major industrial users. The Sangamon River immediately below Lake Decatur is influenced by impoundment, altered flow regime, and point source discharges.

Due to multiple anthropogenic influences, the biotic integrity of the Sangamon River is in constant flux. An intensive sampling program began in 1998-99 and continued through 2015 to document temporal and spatial heterogeneity of an 8.5 km urban reach of the Sangamon River. Two new sampling sites were added starting in June 2015 to include a larger reach of the river. Sampling began directly below the Lake Decatur Dam and continued downstream to incorporate discharges from the Sanitary District of Decatur. These studies (Fischer & Pederson 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014) were intended to characterize stream habitat quality and assess impacts from ongoing reservoir and urban management by evaluating biotic integrity at various trophic levels in the context of the physical and chemical nature of the Sangamon River.

Original sampling locations were associated with operation of the Sanitary District of Decatur that were easily identified by landmarks within the city of Decatur, Illinois, USA. Sites were established in 1998 in conjunction with combined sewage overflow (CSO) facilities and the main treatment plant. Sites were located in the mainstem of the Sangamon River extending from directly below the Lake Decatur dam to the Mechanicsburg Road Bridge, located approximately 30 miles west of Decatur. Sites 1, 3, 4, 5, 6, 7, and 8 extend from the dam to directly above the discharge of the main treatment plant in the upstream reach, and sites 9, 11, 12, and 14 extend from the main treatment discharge to a point approximately 8 river miles downstream near Lincoln Trail Homestead State Park. Sites 15 and 16 extend from Mt. Auburn to Mechanicsburg in order to include a more expansive reach of the river.

We sought to assess the water quality, as well as the macroinvertebrate, freshwater mussel, and fish communities of the Sangamon River near Decatur, Illinois. We sampled the communities in two treatment reaches; one above and one below the Decatur Sanitary District main effluent. Although all of these metrics individually provide some measure of habitat, the combination of all data will provide a broader analysis of the quality of system. Both biotic and abiotic assessments of a given resource are used to determine how similar habitats are compared to their potential. The goal is to identify any factors affecting this ratio as targets for remediation. The analysis may include not only historic ecological indices of multiple trophic levels of biota, but economic and recreational value of an aquatic system as well. For example, although sportfish make up a small portion of the fish assemblage they almost always have the greatest economic and recreational value. As top predators, they may reflect changes in lower trophic levels, but many are not especially sensitive to water quality. In contrast, Unionid mussels have shown sensitivity to various assaults on lotic systems. Mussels can be affected by substrate type and flow

(Harman 1972; Strayer 1983; Vaughn 1997; Watters 1999), and can be harmed by excessive concentrations of heavy metals, phosphorus, and nitrogen (Beckvar *et al.* 2000; Jacobson et al 1997; Mummert et al 2003; Wang et al 2007). As such, the U.S. Environmental Protection Agency proposed using mussel communities in setting ammonia standards (Great Lakes Environmental Center 2005). Based on this information it is important to include these specific communities in assessment of aquatic systems. Macroinvertebrates represent a diverse assemblage occupying multiple habitats in aquatic systems. Often these organisms will display preference towards certain habitat types and physical characteristics which can be a substantial factor in assemblage composition (Álvarez-Cabria et al. 2011, Jowett et al. 1991). This is something that should be taken into account when sampling for these types of communities. They also have shorter life spans and are less mobile than fishes. Thus they can offer more detailed insight into short term impacts or microhabitat specific concerns.

METHODS

Water Data Collection and Chemistry Determination

We collected water quality data monthly from April 2016 to March 2017. Sampling began downstream of the Lake Decatur Dam at the Oakland CSO and proceeded downstream. In the field, we used a YSI ProDSS handheld meter to measure dissolved oxygen, temperature, and specific conductivity, pH, and chloride. In June 2015 we began collecting water samples that were delivered to the SDD for nickel quantification. Water samples were collected just below the surface, returned to the lab on ice, and analyzed within accepted time limits. All analyses followed the Standard Methods for Examination of Water and Wastewater (APHA, 1995).

Total oxidized nitrogen (NO_2-N + NO_3-N) was determined using the cadmium reduction method, and ammonia nitrogen was determined with the phenate method. We used the ascorbic acid method to determine total phosphorus (following persulfate digestion). A Thermo Scientific Evolution 300 UV-VIS spectrophotometer was used for all colorimetric nutrient analyses. Hardness and alkalinity were measured using titration to colorimetric endpoint methods. We considered quality control procedures during all analyses, including but not limited to parallel analyses of laboratory standards.

We calculated and report the averages of each variable for the upstream and downstream reaches. In order to determine overall differences between reaches, principal components analysis (PCA) was conducted for 11 variables after individually log transforming and normalizing the data. Variables that were highly correlated to another and thus redundant were eliminated from the analysis. Correlation analysis was performed to determine the relationship among measured and derived variables.

Habitat Assessment

We assessed physical parameters using a modified Ohio's Quality Habitat Evaluation Index (QHEI) (Rankin 1996) for seven sites (3, 5, 7, 9, 12, 14, and 15). Each 100 m site was divided into three evenly-spaced transects. We measured substrate type and depth every two feet along the width of each transect. Between each transect, we estimated the percent of each instream cover type, the channel morphology, the amount of riparian zone and bank erosion, the pool and riffle quality, and gradient. Each site was scored making a total possible maximum score of 100.

Assessment of Macroinvertebrate and Freshwater Mussel Communities

Macroinvertebrate assemblages were sampled during summer of 2016 from microhabitats present at seven sites in the Sangamon. Three sites (3, 5, 7) were upstream of the effluent discharge of Decatur's sanitary district but below the dam of Lake Decatur and four (9,12, 14, 15) were below the discharge up to 25 miles downstream. Each site was approximately 100 meters in length. Five microhabitat types were sampled, if present, at all sites with three replicates per sample. These microhabitats included riffles, leaf packs, root wads, snags and fine sediments. Three sub samples were taken from each microhabitat type per site. Sampling procedures varied by microhabitat type and were done following the methods described by the EPA macroinvertebrate multihabitat sampling protocol (EPA 2012). Sampling fine sediments or riffle microhabitats included "jabs" with an 18 inch square frame dipnet. A two foot section was sampled from rootwad and snag microhabitats. Leaf pack samples were taken from areas less than half the size of the dipnet.

Artificial samplers were also utilized during this sampling period. These included Hester-Dendy multi-plate samplers and artificial leaf packs. Four of each sampler type were deployed at each site and left submerged for four weeks. Three out of each of the four samplers at every site were collected for processing. Contents from each jab/sampler were concentrated using a bucket sieve, individually placed in a sampling jar, and preserved by addition of 95% ethanol. These were labeled with the site number, microhabitat type, and any unique details about the sampled habitat, and taken back to EIU for processing and identification.

Processing and Identification

In the lab, we subsampled macroinvertebrates from each site using a 30 grid tray. Grids were selected at random until a target of at least 200 macroinvertebrates per microhabitat/artificial sample were picked with a minimum of three grids (10% of the sample) selected, plus any large or rare taxa. Most individuals were identified to genus (or tribe/subfamily for chironomidae). Voucher specimens were catalogued into the EIU invertebrate collection.

We assessed the relative abundance, taxonomic richness, Simpson's diversity (D), percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, and macroinvertebrate index of biotic integrity (MBI) based on taxon-specific environmental sensitivity values provided by the Illinois EPA (Lin & Lee 2007). The Simpson's diversity (D) was calculated using the formula:

$$D = \frac{1}{\Sigma p_i^2}$$

where p_i = is the proportion of the total number of individuals comprised by species *i.*

We also calculated a multihabitat 20-jab method sample (as per USEPA's Rapid Bioassessment Protocol Barbour et al. 1999) for each site. We used the data collected for QHEI per site to determine the proportion of substrate makeup and distributed the collected sample data from each microhabitat type through randomizing selection from each subset. We used a two way MANOVA to assess differences in microhabitat type and the potential difference in equivalent microhabitats and 20 jab samples upstream as opposed to downstream sites. Non-Metric Multidimensional Scaling (NMDS) with adonis for detecting significant differences (vegan package for R) was done to assess communities on an assemblage level. Analyses were performed using Rstudio (version 3.3.3; R Foundation for Statistical Computing 2017).

Freshwater Mussel Communities

Mussel assemblages were sampled during the early fall of 2016 using timed hand searches. We sampled one site (Site 7) below the dam and above the effluent and four sites (12,14,15,16) below the effluent. Five people spread out and searched within the 100 m site at random for 20 minutes. Searches were conducted visually and tactilely (except for one person who used a clam rake). All mussels were collected in mesh bags and identified to species according to Cummings and Mayer (1992). We took length measurements in the field and returned all live mussels to the river.

Assessment of Fish Community

We sampled fish communities at three sites upstream of the SDD (Sites 1, 5, and 8) and three sites downstream of the SDD (Sites 9, 11, and 12) on 29 November 2016 using pulsed DC electrofishing (60 Hz, 25% duty cycle). Each site was sampled for a total effort of 30 minutes. An estimate of relative density was calculated as catch per unit effort (CPUE) as number of fish captured per hour. Fish were identified, weighed (g), measured (mm), and released. All fish that could not be identified in the field were immediately euthanized in formalin and taken back to the laboratory for identification.

River Carpsucker *(Carpiodes carpio)*, Shorthead Redhorse (*Moxostoma macrolepidotum*), and Smallmouth Buffalo (*Ictiobus bubalus*) were sampled in Spring 2016 to assess fin morphologies and determine if feminization of male fishes is occurring. Fishes were collected using pulsed DC electrofishing between sites 11 and 12. Fish were identified, weighed (g), measured for total length and standard length (mm), sexed, and had scales removed. All females and juveniles were released. All males had blood drawn and were euthanized for gonad and otolith removal in the lab. Scales and otoliths were processed for aging. ELISA kits were used to analyze blood plasma for the presence of vitellogenin, the egg-yolk precursor protein. Gonad samples were processed and analyzed for the presence of testicular oocytes. Total length (TL) and standard length (SL) were used to quantify caudal fin morphologies. The length of the caudal fin was quantified by subtracting the SL from the TL. This number was taken as a percentage of the TL to determine the percent of the body that the caudal fin comprised. We compared this metric for these three species from the Sangamon River to populations from a reach of the Embarras River not exposed to a similar wastewater treatment effluent.

RESULTS

Water Data Collection and Chemistry Determination

A total of 11 water quality variables were determined for six sites along the Sangamon River (Table 1). Components 1 and 2 account for a total of 74.8% of the variation in the data during the sampling period. Discrete sampling events (site) cluster on the basis of stream reach (Figure 1). Variables included in the analysis were temperature, dissolved oxygen, pH, conductivity, chloride, hardness, total alkalinity, total oxidized nitrogen, ammonia, total phosphorus, and nickel. Chloride and nickel were dropped due to multicollinearity. Variation in component 1 is largely due to temperature ($r = 0.931$) and dissolved oxygen ($r = -0.273$), whereas factor 2 is heavily influenced by total phosphate ($r = 0.7199$), total alkalinity ($r = 0.473$), and specific conductivity ($r = 0.468$). Samples collected from the downstream and upstream were significantly different (ANOSIM, Global $R = 0.340$, $p < 0.001$).

Spearman correlation analysis was used to assess the relationship between nickel concentrations and river discharge. Soluble nickel was detected at all sites for at least one month from April 2016 - March 2017. Analyses revealed strong negative correlations between soluble nickel concentrations and river discharge. Spearman correlation analysis was statistically significant ($p= 0.0002$). Linear Regression was used to further assess the relationship between river discharge and nickel concentrations for sites 9 and 12, the two sites closest to the SDD main treatment facility. Following removal of outliers for normality, a significant negative relationship was found ($p= 0.001$) (Figure 2). Lowest nickel concentrations were seen at peak river discharge, but nickel concentration varied extensively at low flow.

Habitat Assessment

QHEI scores ranged from poor to excellent. Higher QHEI scores were typical of sites farther downstream (Figure 3).

Assessment of Macroinvertebrate and Freshwater Mussel Communities

A total of 58 different taxa were identified from the seven sites sampled (Tables 4 & 5). When comparing overall assemblages there was no significant difference between the 2 reaches for Simpson's

Diversity (*p* = 0.159) (Figure 4). However, estimated abundance, richness, percent EPT, EPT richness, and MBI was significantly higher (lower for MBI) downstream of SDD main outfall (estimated abundance *p* = 0.006, richness *p* = <0.001, percent EPT *p* = <0.001, EPT richness *p* = <0.001, MBI *p* = <0.001) (Figure 4). When comparing equivalent microhabitat types by reach, however, only root wads, sediments and 20 jab samples were significantly different (rootwads for MBI *p adj.* = 0.004, sediments for percent EPT *p adj.* = 0.017, 20 jab for estimated abundance *p adj.* = <0.001) (Table 7). Although sites appeared distinct on NMDS (Figure 5), assemblages from the 20 jab samples were not significantly different between reaches. Significant differences in assemblages between microhabitat types were seen with all indices (*p* = <0.05). Notable differences between specific microhabitat types are summarized in Table 6.

MBI scores ranged between <5 ("Excellent") to >7.5 ("very poor"). Midges were abundant in both reaches while Hydropsychid caddisflies were much more abundant in the downstream reach but still had a strong presence upstream. Taxa unique to the upstream reach included operculate snails and planorbid snails. Taxa unique to the downstream reach include dobsonflies, stoneflies, caddisflies belonging to the families hydroptilidae and philopotamidae, and other taxa (Tables 4 & 5).

Freshwater Mussels

High water levels prevented adequate sampling on several scheduled trips when sampling crews were available. Sampling occurred in early fall 2016, but the water level was still above baseflow, preventing access to pools where mussels concentrate during low flow. Only two live mussels were collected during the entirety of the sampling. Mussel sampling will be conducted in 2017 and 2018.

Assessment of Fish Community

Pulsed DC electrofishing was conducted at six sites for 30 minutes each: 3 upstream and 3 downstream of the Sanitary District of Decatur. We sampled a total of 179 individuals from 21 species (Table 8). The most dominant family sampled was Catostomidae and comprised over 56% of the total sample. The sportfish community in the Sangamon River comprised a small percentage of the total sample and included White Bass (*Morone chrysop*s), Black Crappie (*Pomoxis nigromaculatus*), Sauger (*Sander canadensis*), and Largemouth Bass (*Micropterus salmoides*), (Table 8). The non-sportfish community was dominated by Buffalo species *(Ictiobus sp.)* and Gizzard Shad *(Dorosoma cepedianum)* (Table 8). Relative density, as estimated by catch per unit effort (CPUE) in fish per hour, was highest in the upstream reach at Site 5 and lowest in the downstream reach at Site 9 (Table 9).

River Carpsucker, Shorthead Redhorse, and Smallmouth Buffalo were sampled in spring 2016 to assess fin morphologies and the reproductive health of male fishes. ELISA kits were used to analyze blood plasma for the presence of vitellogenin. Vitellogenin, the egg-yolk precursor protein only found in adult female fishes, is a common biomarker to determine the occurrence of feminization. Fish testes were dissected, histologically prepared, and analyzed for the presence of testicular oocytes, another indication of feminization. A high percentage of fishes exhibiting feminization can be caused by exposure to endocrine disrupting compounds in a waterway. No testicular oocytes were found in the fishes sampled from the Sangamon River. Vitellogenin analyses yielded inconclusive results. Low levels of vitellogenin were detected in a high percentage of all three species. Further analyses will be conducted to confirm these results.

Total length (TL) and standard length (SL) were used to quantify fin morphologies. The length of the caudal fin was taken as a percentage of the TL to determine the percent of the total body length that the caudal fin comprised. We compared this metric for these three populations from the Sangamon River to populations from the Embarras River. River Carpsucker and Smallmouth Buffalo from the Sangamon River had caudal fins comprising a significantly higher percent of the total body length compared to those from the Embarras River (Table.10). No difference was found for Shorthead Redhorse. Significantly longer caudal fins cause River Carpsucker and Smallmouth Buffalo from the Sangamon River to have smaller standard lengths compared to their total length than fishes from the Embarras River (Figure 7).

DISCUSSION

The primary differences between the upstream and downstream reaches are likely attributable to metrics related to reservoir discharge and inputs from the SDD main discharge. Outflow from Lake Decatur is the primary input to the upstream reach, which is compromised as a result of management to maintain reservoir levels by eliminating outflow. The discharge from the main treatment plant of the SDD alters instream water chemistry, especially during periods of low reservoir discharge. Consistent flow downstream of the SDD's main outfall during low discharge periods may help maintain physical habitat quality while the upstream reach becomes disconnected pools. A significant negative Spearman correlation was found between river discharge and nickel concentrations, indicating lower nickel concentrations during periods of high flow. These relationships may be of biological importance and agree with other water chemistry patterns. Future analyses should take into consideration the volume of reservoir outflow and discharge from the SDD's main outfall, as well as nickel concentration in the effluent stream, during the time of water sample collection.

The macroinvertebrate communities sampled, especially above and immediately downstream the effluent, were heavily dominated by aquatic midges. Midges are common in organic rich habitats and are often the most abundant taxa in these habitats (Rabeni and Wang 2001). Most metrics commonly used to describe water quality (e.g., taxa richness, MBI), using macroinvertebrate assemblages, were significantly different between reaches sampled. However, Simpson's diversity, which is weighted toward abundance of the most dominant species, was not significant. Macroinvertebrate assemblages varied between microhabitats, and it appeared that the communities from the same microhabitats also differ between upstream and downstream reaches (Figures 4 & 5). This may be due to different availability of these physical habitats between sites, as is shown by QHEI scores (Figures 3). Although richness (overall and EPT) was higher when comparing the pooled 20 jab sample data to all the other microhabitat types, there were no significant differences in the more proportionally-based indices (percent EPT and MBI) (Figures 4 & 6). Even though the 20 jab samples generally have higher richness than any of the other sample types (Figure 6), combined riffle and rootwad samples would essentially yield similar results to that of the 20 jab samples. The multihabitat 20 jab method may not be necessary to sample the Sangamon River adequately for macroinvertebrates. Overall patterns of %EPT (a measure of sensitive taxa) were similar with all sampling except artificial leaf packs, and all were dominated by the most tolerant EPT taxa. Riffle samples most closely matched the 20 jab samples, but they are rarely found above the effluent outflow. Rootwads were present at every site, and typically harbored the most high quality taxa, as measured by MBI, richness and non-hydropsychidae/polycentropodidae percent EPT taxa (Figures 4 & 6). Although rootwad microhabitats can vary greatly between sites, they are an important habitat for sampling in the Sangamon River. Sampling riffles, where present, and rootwads at all sites should reflect patterns seen using 20 jab methods and should increase the likelihood of collecting rare or sensitive taxa.

Fall sampling of fish communities in 2016 resulted in the collection of species that were not collected in spring 2015. The fall 2016 sample also lacked several species collected in spring 2015. These differences may be due to seasonal variation in the fish communities or due to abiotic factors, such as variation in water level. Future, more extensive sampling using multiple gears or more frequent sampling will provide a more accurate representation of the fish species composition. These data will further attempt to determine any environmental impacts and the best management strategies for fish species in this portion of the Sangamon River.

While low levels of vitellogenin were detected in a high percentage of all three species, these concentrations do not appear to be environmentally relevant, especially when considering the absence of testicular oocytes. River Carpsucker and Smallmouth Buffalo from the Sangamon River had caudal fins comprising a significantly higher percent of the total body length compared to those from the Embarras River. No difference was found for Shorthead Redhorse. The elongation of the caudal fin in these fishes causes inaccurate calculations of relative weight, a condition metric that incorporates total length. Elongated caudal fins cause these fishes to appear in worse somatic condition. The cause and impacts of these elongated fins remains unclear. Further investigation into elongated fins and how they may affect these fishes will continue in 2017.

Sampling of Unionid mussel populations will continue during Summer 2017 when the river returns to baseflow.

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TABLES AND FIGURES

Table 1. Measured water quality variables for six Sangamon River sites associated with the Sanitary District of Decatur. Variables below the detection limit are indicated as 0.00. Missing data are indicated by blank cells.

Date	Gage Height (ft)	Discharge (cfs)	Site	DO (mg/L)	Temp (C)	pH	Spec. Cond	Hardnes (mg/L)	Total Alk (mg/L)	NO2/NO3 (mg/L)	NH4 (mg/L)	PO4 Total (mg/L)	Nickel (mg/L)	Chloride (mg/L)
4/21/2016	5.22	514	5	10	16.1	8.33	556	394.2	167.52	6.116769	0	0.135638	0.005	24.81
5/17/2016	11.1	2720	5	10	15.6	8.55	550	332.15	69.8	20.2774	0.01557664	0.456279	0.003	15.05
6/22/2016	3.85	225	5	7	26.6	8.65	529	372.3	97.72	15.1321	0	0.691974	0	123.49
7/18/2016	3.59	183	5	7	27.6	8.47	506	481.8	795.72	6.90977	0.05581307	0.668757	0	118.3
8/16/2016	5.75	645	5	7	26.6	8.66	423	405.15	167.52	3.580008	0.0890157		0	129.71
9/21/2016	4.17	392	5	8	25	8.56	474	248.2	237.32	3.790644	0	0.258474	0	100.45
10/19/2016	3.15	159	5	8	18.4	8.48	497	281.05	209.4	2.061669	0.06194837	0.076616	0	86.22
11/16/2016	2.84	88	5	10	11.3	8.7	532	226.3	265.24	3.457369	0.03329039	0.220479	0	11.44
12/15/2016	5.37	533	5	15	1.2	8.79	623	332.15	307.12	7.66245	0	0.168637	0	6.15
1/17/2017	5.82	645	5	15	1.6	8.61	662	328.5	251.28	9.731601	0		0	8.1
2/15/2017	4.3	317	5	14	3.9	9	619	317.55	237.32	5.92106	0.00398425	0.084837	0	8.81
3/21/2017	2.58	63	5	11	7.1	8.8	581	372.3	223.36	4.617505	0.09789815	0.100344	0	26.21
4/21/2016	5.22	514	9	9	17.3	8.2	864	346.75	195.44	6.266463	0.01090158	1.594182	0.007	87.72
5/17/2016	11.1	2720	9	10	16	8.44	696	332.15	111.68	12.57553	0.04258607	0.864332	0.003	41.85
6/22/2016	3.85	225	9	5	26.4	8.12	1283	343.1	223.36	36.56944	0.02235224	4.434819	0.007	584.13
7/18/2016	3.59	183	9	6	27.7	8.3	839	390.55	209.4	5.816971	0.07273752	2.294867	0.003	353.67
8/16/2016	5.75	645	9	7	26.6	8.51	700	186.15	167.52	2.396873	0.0748385	1.75101	0.004	318.48
9/21/2016	4.17	392	9	7	25.6	8.4	829	229.95	251.28	2.911842	0.02589732	1.975989	0.004	428.71
10/19/2016	3.15	159	9	8	20.1	8.29	1216	397.85	237.32	2.871799	0.06077833	3.974467	0.005	545.32
11/16/2016	2.84	88	9	9	15	8.34	1532	383.25	376.92	4.758363	0.01468992	7.32241	0.008	57.85
12/15/2016	5.37	533	9	14	3	8.62	939	295.65	307.12	6.853048	0	4.038498	0.004	19.38
1/17/2017	5.82	645	9	14	4.1	8.6	1026	324.85	279.2	6.511156	0	2.261658	0.003	23.92
2/15/2017	4.3	317	9	13	6.2	8.84	1032	313.9	293.16	6.926993	0	2.676358	0.003	20.58
3/21/2017	2.58	63	9	9	15.7	8.15	2591	459.9	530.48	9.447961	0.16998441	11.38262	0.009	274.48
4/21/2016	5.22	514	12	9	16.8	8.18	702	412.45	223.36	7.688559	0	0.992721	0.004	52.7
5/17/2016	11.1	2720	12	10	15.4	8.52	604	288.35	139.6	15.04594	0.02638041	0.629523	0.003	25.18
6/22/2016	3.85	225	12	6	26.3	8.13	1127	302.95	223.36	22.63517	0	3.892869	0.005	406.53
7/18/2016	3.59	183	12	6	27.6	8.22	928	576.7	474.64	5.423563	0.04251528	2.826772	0.004	446.67
8/16/2016	5.75	645	12	7	26.2	8.5	506	204.4	139.6	3.927989	0.09256	0.662653	0.003	214.36
9/21/2016	4.17	392	12	7	25.2	8.42	688	237.25	251.28	2.179506	0.01415075	1.522756	0.003	290.72
10/19/2016	3.15	159	12	7	19.6	8.33	1030	427.05	279.2	3.573912	0.06779859	3.941519	0.004	468.88
11/16/2016	2.84	88	12	9	13.8	8.37	1484	354.05	390.88	6.384606	0.01572328	7.474861	0.007	55
12/15/2016	5.37	533	12	14	1.5	8.65	763	313.9	293.16	6.313447	0.04752942	1.316339	0	11.23
1/17/2017	5.82	645	12	14	3.2	8.51	833	324.85	293.16	6.986903	0	1.080754	0	18.73
2/15/2017	4.3	317	12	13	5.1	8.84	882	324.85	293.16	5.061382	0.01875431	2.112774	0	16.05
3/21/2017	2.58	63	12	9	14.3	8.23	2452	368.65	502.56	9.52115	0.11766374	11.12218	0.009	182.08
4/21/2016	5.22	514	14	8	17.2	8.1	920	346.75	181.48	9.457816	0.13393442	1.61824	0.005	101.89
5/17/2016	11.1	2720	14	10	15.6	8.51	606	299.3	139.6	11.99426	0.0601422	0.567957	0.003	31.64

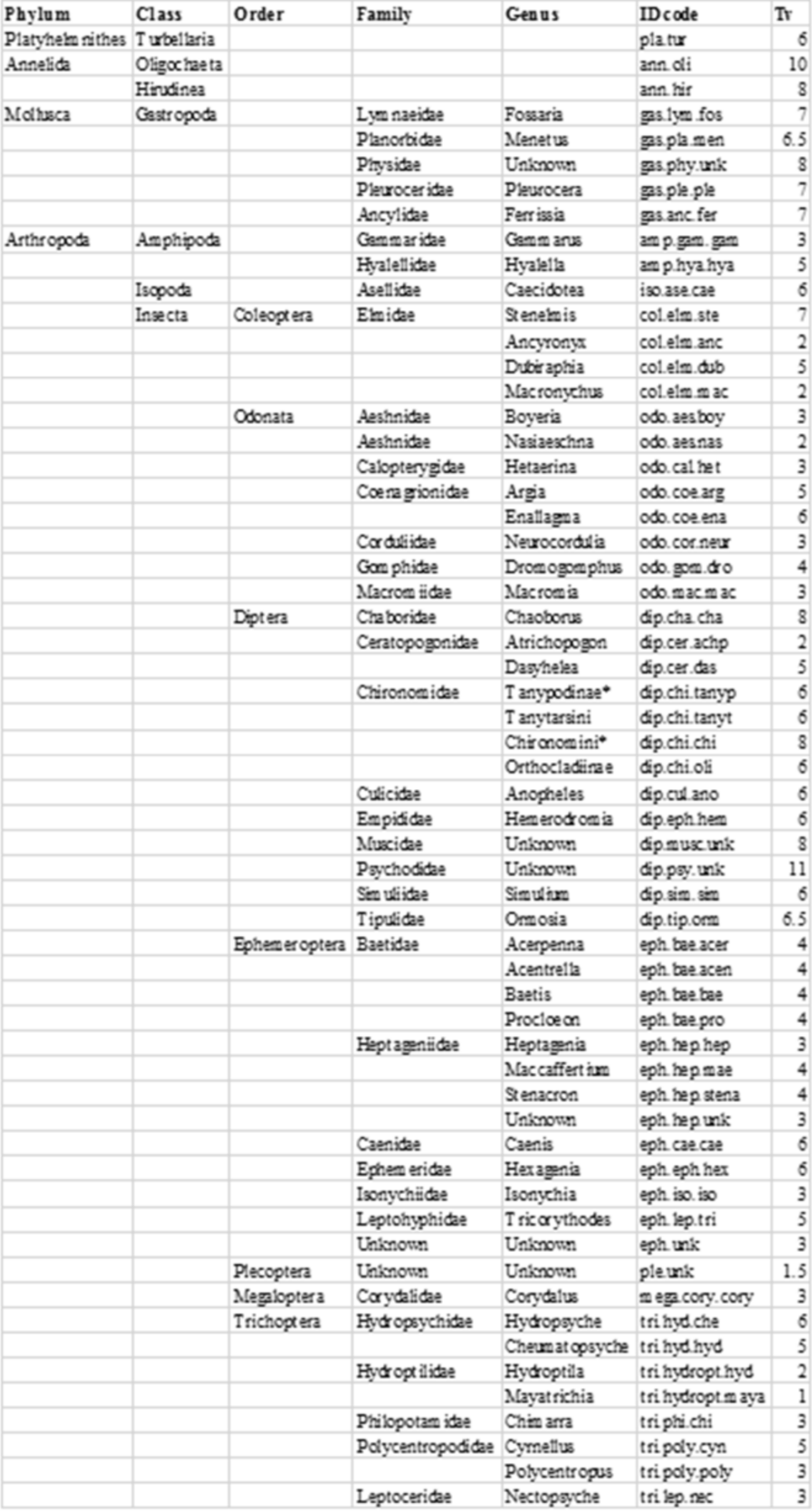
6/22/2016	3.85	225	14	6	26.5	8.22	928	343.1	209.4	7.986317	0	1.996042	0.004	418.28
7/18/2016	3.59	183	14	7	27.6	8.23	932	397.85	307.12	5.161291	0.01350193	3.328283	0.004	471.61
8/16/2016	5.75	645	14	7	25.7	8.49	508	204.4	97.72	0.587372	0.04175835	1.161137	0.003	226.85
9/21/2016	4.17	392	14	7	24.7	8.36	827	255.5	265.24	3.790644	0.03411992	1.92828	0.006	395.77
10/19/2016	3.15	159	14	7	19.8	8.29	1094	328.5	293.16	2.250699	0.02918714	4.073313	0.005	516.61
11/16/2016	2.84	88	14	9	12.1	8.3	1640	343.1	390.88	6.774904	0.01468992	8.328589	0.008	63.09
12/15/2016	5.37	533	14	14	0.8	8.6	776	357.7	307.12	7.212783	0.03478723	1.433827	0	10.49
1/17/2017	5.82	645	14	13	3.9	8.46	935	335.8	307.12	6.072004	0.00672094	1.461179	0	25.84
2/15/2017	4.3	317	14	13	5.2	8.83	935	321.2	307.12	8.193725	0	2.323067	0.002	15.98
3/21/2017	2.58	63	14	9	12.6	8.19	2416	427.05	502.56	13.03421	0.07348184	12.48948	0.008	171.46
4/21/2016	5.22	514	15	9	16.1	8.16	724	321.2	181.48	5.218603	0	0.839348	0.004	44.25
5/17/2016	11.1	2720	15	10	15.3	8.46	614	299.3	153.56	17.37104	0.02773088	0.54648	0.003	28.44
6/22/2016	3.85	225	15	6	26.3	8.28	865	306.6	195.44	12.98836	0.94956553	1.555708	0.004	312.74
7/18/2016	3.59	183	15	7	27.2	8.22	889	423.4	279.2	3.675084	0	2.416445	0.004	424.14
8/16/2016	5.75	645	15	6	25.2	8.21	1147	350.4	195.44	3.510412	0.14099879	4.442826	0.008	542.15
9/21/2016	4.17	392	15	7	24.3	8.29	999	262.8	349	4.083579	0	2.643911	0.005	493.81
10/19/2016	3.15	159	15	7	19.2	8.26	951	295.65	265.24	2.250699	0	3.644982	0.004	447.33
11/16/2016	2.84	88	15	9	10.4	8.34	1516	365	349	6.384606	0	6.849811	0.008	49.68
12/15/2016	5.37	533	15	14	0.3	8.59	793	244.55	307.12	7.167816	0.02204505	0.982151	0	10.25
1/17/2017	5.82	645	15	14	3.5	8.44	831	328.5	293.16	6.437964	0	1.10453	0	23.1
2/15/2017	4.3	317	15	13	4.9	8.88	890	313.9	279.2	7.448589	0	1.767895	0	18.86
3/21/2017	2.58	63	15	11	10.8	8.36	1767	368.65	404.84	7.325488	0	4.538498	0.006	116.1
4/21/2016	5.22	514	16	9	16.4	8.11	712	302.95	181.48	8.073144	0	0.72507	0.004	45.59
5/17/2016	11.1	2720	16	9	15.1	8.27	625	292	153.56	8.361299	0.0317823	0.588001	0.004	13.55
6/22/2016	3.85	225	16	7	27	8.3	813	328.5	209.4	11.02327	0.18991311	1.454092	0.004	362.54
7/18/2016	3.59	183	16	7	27.1	8.14	867	368.65	139.6	1.926605	0.02921749	2.340459	0.004	415.67
8/16/2016	5.75	645	16	7	23.5	8.2	1113	259.15	223.36	3.162431	0.06656846	4.002498	0.009	423
9/21/2016	4.17	392	16	7	24.3	8.38	833	255.5	293.16	2.325973	0	1.824911	0.004	414.63
10/19/2016	3.15	159	16	8	19.1	7.8	1431	273.75	321.08	2.493738	0	4.831128	0.007	741.6
11/16/2016	2.84	88	16	10	9.5	8.47	1041	419.75	362.96	4.237966	0	1.616933	0.005	15.27
12/15/2016	5.37	533	16	14	0	8.59	781	321.2	321.08	8.741653	0.02088667	1.405345	0	10.15
1/17/2017	5.82	645	16	13	3.5	8.35	847	335.8	307.12	5.669448	0	0.953945	0	17.72
2/15/2017	4.3	317	16	12	5.1	8.96	856	310.25	293.16	7.932927	0	1.557603	0	17.81
3/21/2017	2.58	63	16	10	10.7	8.5	1298	401.5	404.84	8.130564	0	2.674743	0.004	82.16
Upstream	mean			10	15.0833	8.63	546	340.97	252.443	7.438195	0.02979388	0.286203	7E-04	54.895
Downstream	mean			9	15.92	8.38	1021	330.81	274.314	7.285097	0.0476479	2.969394	0.004	207.74

Table 2. Monthly nickel values for 6 Sangamon River sites associated with the Sanitary District of Decatur. Dates range from April 2016 to March 2017. Values <0.0024 indicate values below the detection limit. River discharge in cubic feet per second at the Route 48 bridge gauge at time of sampling is also recorded.

Nickel Concentration (mg/L)

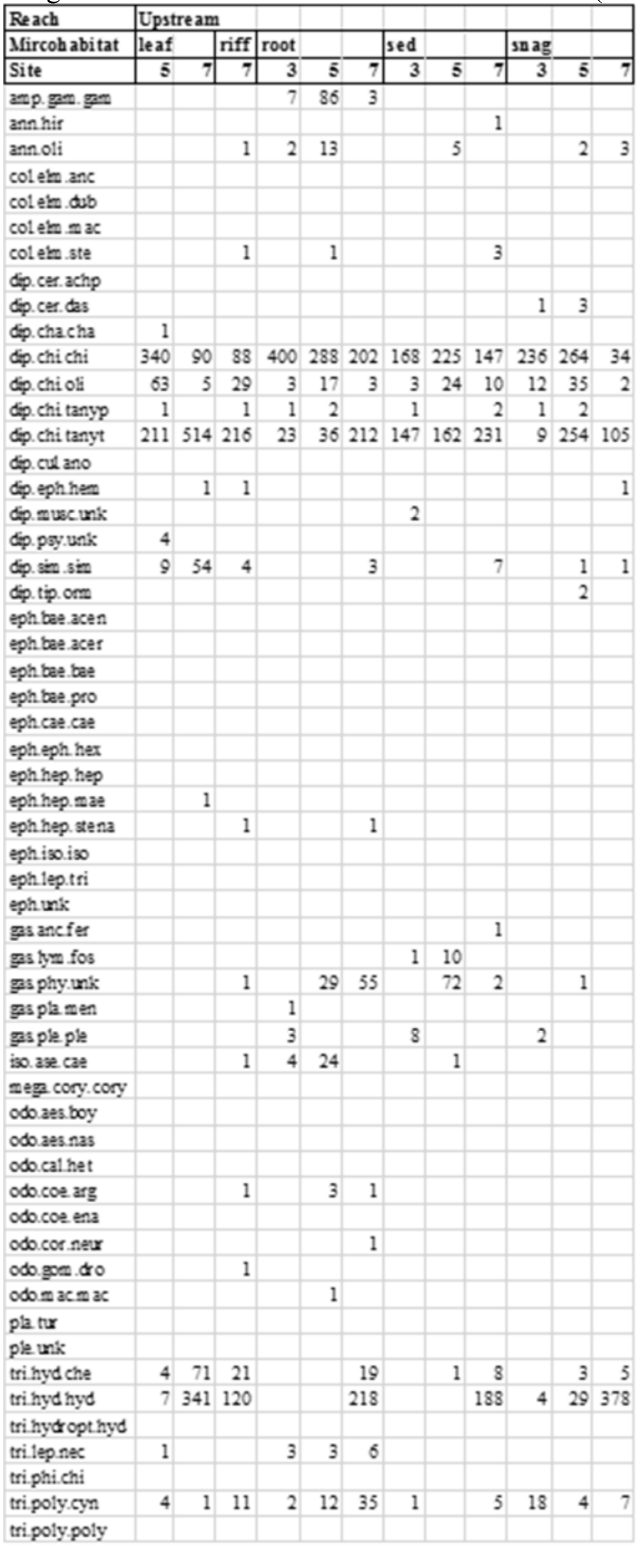
	5	9	12	14	15	16	River discharge (cfs)
April	0.00491	0.00724	0.00363	0.00505	0.00359	0.00362	514
May	0.00294	0.00348	0.00327	0.00301	0.00317	0.00416	2720
June	<0.0024	0.00665	0.00544	0.00443	0.00393	0.00405	225
July	<0.0024	0.00297	0.00424	0.00435	0.00424	0.00446	183
August	<0.0024	0.00354	0.00257	0.00347	0.00795	0.00896	645
September	<0.0024	0.00376	0.00291	0.00561	0.00483	0.00381	392
October	<0.0024	0.00472	0.00429	0.00519	0.00439	0.00651	159
November	<0.0024	0.00808	0.00732	0.00826	0.00768	0.00533	88
December	<0.0024	0.00363	<0.0024	<0.0024	<0.0024	<0.0024	533
January	<0.0024	0.00263	<0.0024	<0.0024	<0.0024	<0.0024	645
February	<0.0024	0.00312	<0.0024	0.00249	<0.0024	<0.0024	317
March	<0.0024	0.00927	0.00874	0.00844	0.00569	0.00395	2.58

Table 3. List of macroinvertebrate taxa found in the Sangamon River during the summer of 2016. This includes individual identification codes and tolerance values (Tv) ranging from 0 (intolerant) to 11 (tolerant).



Phylum	Class	Order	Family	Genus	IDcode	Tv
Platyhelminthes	Turbellaria				pla.tur	6
Annelida	Oligochaeta				ann.oli	10
	Hirudinea				ann.hir	8
Mollusca	Gastropoda		Lymnaeidae	Fossaria	gas.lym.fos	7
			Planorbidae	Menetus	gas.pla.men	6.5
			Physidae	Unknown	gas.phy.unk	8
			Pleuroceridae	Pleurocera	gas.ple.ple	7
			Ancylidae	Ferrissia	gas.anc.fer	7
Arthropoda	Amphipoda		Gammaridae	Gammarus	amp.gam.gam	3
			Hyalellidae	Hyalella	amp.hya.hya	5
	Isopoda		Asellidae	Caecidotea	iso.ase.cae	6
	Insecta	Coleoptera	Elmidae	Stenelmis	col.elm.ste	7
				Ancyronyx	col.elm.anc	2
				Dubiraphia	col.elm.dub	5
				Macronychus	col.elm.mac	2
		Odonata	Aeshnidae	Boyeria	odo.aes.boy	3
			Aeshnidae	Nasiaeschna	odo.aes.nas	2
			Calopterygidae	Hetaerina	odo.cal.het	3
			Coenagrionidae	Argia	odo.coe.arg	5
				Enallagma	odo.coe.ena	6
			Corduliidae	Neurocordulia	odo.cor.neur	3
			Gomphidae	Dromogomphus	odo.gom.dro	4
			Macromiidae	Macromia	odo.mac.mac	3
		Diptera	Chaboridae	Chaoborus	dip.cha.cha	8
			Ceratopogonidae	Atrichopogon	dip.cer.achp	2
				Dasyhelea	dip.cer.das	5
			Chironomidae	Tanypodinae*	dip.chi.tanyp	6
				Tanytarsini	dip.chi.tanyt	6
				Chironomini*	dip.chi.chi	8
				Orthocladiinae	dip.chi.oli	6
			Culicidae	Anopheles	dip.cul.ano	6
			Empididae	Hemerodromia	dip.eph.hem	6
			Muscidae	Unknown	dip.musc.unk	8
			Psychodidae	Unknown	dip.psy.unk	11
			Simuliidae	Simulium	dip.sim.sim	6
			Tipulidae	Ormosia	dip.tip.orm	6.5
		Ephemeroptera	Baetidae	Acerpenna	eph.bae.acer	4
				Acentrella	eph.bae.acen	4
				Baetis	eph.bae.bae	4
				Procloeon	eph.bae.pro	4
			Heptageniidae	Heptagenia	eph.hep.hep	3
				Maccaffertium	eph.hep.mae	4
				Stenacron	eph.hep.stena	4
				Unknown	eph.hep.unk	3
			Caenidae	Caenis	eph.cae.cae	6
			Ephemeridae	Hexagenia	eph.eph.hex	6
			Isonychiidae	Isonychia	eph.iso.iso	3
			Leptohyphidae	Tricorythodes	eph.lep.tri	5
			Unknown	Unknown	eph.unk	3
		Plecoptera	Unknown	Unknown	ple.unk	1.5
		Megaloptera	Corydalidae	Corydalus	mega.cory.cory	3
		Trichoptera	Hydropsychidae	Hydropsyche	tri.hyd.che	6
				Cheumatopsyche	tri.hyd.hyd	5
			Hydroptilidae	Hydroptila	tri.hydropt.hyd	2
				Mayatrichia	tri.hydropt.maya	1
			Philopotamidae	Chimarra	tri.phi.chi	3
			Polycentropodidae	Cyrnellus	tri.poly.cyn	5
				Polycentropus	tri.poly.poly	3
			Leptoceridae	Nectopsyche	tri.lep.nec	3

Table 4. Summary of macroinvertebrates sampled from five natural habitats in seven sites in the Sangamon River in summer 2016. Taxa ID in code (refer to Table 3).



Reach	**Upstream**											
Mircohabitat	**leaf**		**riff**	**root**			**sed**			**snag**		
Site	**5**	**7**	**7**	**3**	**5**	**7**	**3**	**5**	**7**	**3**	**5**	**7**
amp.gam.gam				7	86	3						
ann.hir									1			
ann.oli			1	2	13			5			2	3
col.elm.anc												
col.elm.dub												
col.elm.mac												
col.elm.ste			1		1				3			
dip.cer.achp												
dip.cer.das										1	3	
dip.cha.cha	1											
dip.chi.chi	340	90	88	400	288	202	168	225	147	236	264	34
dip.chi.oli	63	5	29	3	17	3	3	24	10	12	35	2
dip.chi.tanyp	1		1	1	2		1		2	1	2	
dip.chi.tanyt	211	514	216	23	36	212	147	162	231	9	254	105
dip.cul.ano												
dip.eph.hem		1	1									1
dip.musc.unk							2					
dip.psy.unk	4											
dip.sim.sim	9	54	4			3			7		1	1
dip.tip.orm											2	
eph.bae.acen												
eph.bae.acer												
eph.bae.bae												
eph.bae.pro												
eph.cae.cae												
eph.eph.hex												
eph.hep.hep												
eph.hep.mae		1										
eph.hep.stena			1			1						
eph.iso.iso												
eph.lep.tri												
eph.unk												
gas.anc.fer									1			
gas.lym.fos							1	10				
gas.phy.unk			1		29	55		72	2		1	
gas.pla.men				1								
gas.ple.ple				3			8			2		
iso.ase.cae			1	4	24			1				
mega.cory.cory												
odo.aes.boy												
odo.aes.nas												
odo.cal.het												
odo.coe.arg			1		3	1						
odo.coe.ena												
odo.cor.neur						1						
odo.gom.dro			1									
odo.mac.mac					1							
pla.tur												
ple.unk												
tri.hyd.che	4	71	21			19		1	8		3	5
tri.hyd.hyd	7	341	120			218			188	4	29	378
tri.hydropt.hyd												
tri.lep.nec	1			3	3	6						
tri.phi.chi												
tri.poly.cyn	4	1	11	2	12	35	1		5	18	4	7
tri.poly.poly												

Reach	**Downstream**																		
Mircohabitat	**leaf**				**riff**			**root**				**sed**				**snag**			
Site	**9**	**12**	**14**	**15**	**12**	**14**	**15**	**9**	**12**	**14**	**15**	**9**	**12**	**14**	**15**	**9**	**12**	**14**	**15**
amp.gam.gam								5		3									
ann.hir												1		1	1				
ann.oli								1			1		1		17	1			
col.elm.anc									1										
col.elm.dub									3	4	4				1				
col.elm.mac		1							1										
col.elm.ste			2		29	1	18	2		21	2		1		1			2	
dip.cer.achp		1							2										1
dip.cer.das																			
dip.cha.cha																			
dip.chi.chi	154	317	98	19	108	27	31	145	276	84	92	68	18	36	25	33	25	5	56
dip.chi.oli	1	1	2	13	2	51	32			1	4	36		49	13	9		5	11
dip.chi.tanyp	3	3	12	6	12	14	8	2	6	16	11	2		1		5	1	1	4
dip.chi.tanyt	181	18	33	265	137	37	241	13	93	68	176	329	25	69	136	219	102	17	44
dip.cul.ano								1											
dip.eph.hem				1	4	1				2	2			5	2				1
dip.musc.unk																			
dip.psy.unk	1																		
dip.sim.sim	10	3	3	41	1	14	24					8			1	12	2		1
dip.tip.orm																			
eph.bae.acen							1												
eph.bae.acer			2	1		16	3			2	1		2		1				1
eph.bae.bae		14	38	52	7	118	112			15	3				2			43	37
eph.bae.pro																		1	
eph.cae.cae		1					1	1	2										
eph.eph.hex															1				
eph.hep.hep						2	12											2	1
eph.hep.mae			53	6	3	55	54			80	50		1	6	4			2	3
eph.hep.stena		2	5				2	1		3	5								
eph.iso.iso			7			17	23			18	2								
eph.lep.tri			2			24	16			80	8			73	4				
eph.unk															1				
gas.anc.fer								1											
gas.lym.fos																			
gas.phy.unk	1	2					1	74	118		2			1					
gas.pla.men																			
gas.ple.ple																			
iso.ase.cae																			
mega.cory.cory			1				5			1									
odo.aes.boy								1			2								
odo.aes.nas										1									
odo.cal.het											4								
odo.coe.arg		9	1					7	53	76	29								
odo.coe.ena								1			1								
odo.cor.neur									1	1									
odo.gom.dro					1					1									
odo.mac.mac			1					1		5									
pla.tur										2								1	
ple.unk																1			
tri.hyd.che	13		32	4	32	74	48	1	3	17		10		7	10	19	2	2	3
tri.hyd.hyd	39	40	365	267	249	325	559	6	34	396	10	120	26	463	204	265	333	491	236
tri.hydropt.hyd						2													
tri.lep.nec	2	34	5					240	66	106	151			1				1	
tri.phi.chi				1															
tri.poly.cyn		1	1		15			17	20	17						2	3	1	2
tri.poly.poly				2															

Table 5. Summary of macroinvertebrates sampled from two artificial habitats in seven sites in the Sangamon River in summer 2016. Taxa ID in code (refer to Table 3).

Reach	**Upstream**						**Downstream**							
Habitat	**art. leaf**			**hester-dendy**			**art. leaf**				**hester-dendy**			
Site	**3**	**5**	**7**	**3**	**5**	**7**	**9**	**12**	**14**	**15**	**9**	**12**	**14**	**15**
ann.hir									1					
ann.oli	3	29	78				2	16		6				1
col.elm.dub										1				
col.elm.ste		4						1	2	2		1		1
dip.chi.chi	379	329	268	288	434	153	343	200	105	149	309	147	47	205
dip.chi.oli	50	37	17	89	133	73	20	18	18	6	39	28	1	6
dip.chi.tanyp	3	2	13	4	3	19	6	9	30	12	14	15	8	26
dip.chi.tanyt	65	135	57	18	37	42	211	149	369	416	52	167	232	299
dip.eph.hem									2				1	
dip.sim.sim								3		4			2	
eph.bae.bae													1	
eph.cae.cae									1	1				2
eph.eph.hex										1				
eph.hep.mae									1	2			53	19
eph.hep.stena	3							2					3	1
eph.hep.unk	1													
eph.iso.iso									1	1			3	9
eph.lep.tri									7				10	3
gas.lym.fos		1												
gas.phy.unk		1	2		1									
gas.ple.ple	19													
mega.cory.cory													1	2
odo.coe.arg			4					9		3		2	3	12
odo.gom.dro		4			2			2		6				
odo.mac.mac							1							
pla.tur			2										1	
tri.hyd.che	1	3	2		6			3	15	3		1	40	11
tri.hyd.hyd	6	38	4	1	18	16	1	7	59	34		109	176	64
tri.hydropt.maya													2	
tri.lep.nec									5	4			2	
tri.poly.cyn	41	26	39	147	72	273	69	51	15	12	207	141	27	23

Table 6. Adjusted p-values (Bonferroni correction) for comparisons between microhabitat types with regards to population indices. Only habitat comparisons that have significantly different values are displayed. NS means not significant.

Habitat comparisons	MBI	Percent EPT	Est. Abundance	Richness	EPT Richness	Simpson's Diversity
Riffle - Art. Leaf	0.0010068	0.0017016	NS	NS	NS	NS
Rootwad - Art. Leaf	0.047667	NS	NS	NS	NS	NS
Snag - Art. Leaf	0.0013776	0.0019794	NS	NS	NS	NS
Snag - Hester Dendy	NS	NS	NS	NS	NS	0.0009204
Sediment - Riffle	NS	NS	0.0007062	0.0018042	0.0015294	NS
Snag - Riffle	NS	NS	0.0058548	0.0021804	NS	0.0001752
Sediment - Rootwad	NS	NS	NS	0.0019884	NS	NS
Snag - Rootwad	NS	NS	NS	0.0024804	NS	NS

Table 7. Adjusted p-values (Bonferroni correction) for microhabitat type, artificial sampler, and 20 jab samples with regards to comparing upstream vs downstream. NS means not significant.

Sample Type	MBI	Percent EPT	Est. Abundance	Richness	EPT Richness	Simpson's Diversity
20 Jab	NS	NS	0.0000012	NS	NS	NS
Art. Leaf	NS	NS	NS	NS	NS	NS
Hester Dendy	NS	NS	NS	NS	NS	NS
Leaf	NS	NS	NS	NS	NS	NS
Riffle	NS	NS	NS	NS	NS	NS
Rootwad	0.0038544	NS	NS	NS	NS	NS
Sediments	NS	0.0173412	NS	NS	NS	NS
Snag	NS	NS	NS	NS	NS	NS

Table 8. Summary of fish species sampled using pulsed DC electrofishing upstream and downstream of the Sanitary District of Decatur in the Sangamon River on 29 November 2016.

			Site						
			Upstream			Downstream			
Species		**Family**	**1**	**5**	**8**	**9**	**11**	**12**	**Total**
Bighead Carp	*Hypophthalmichthys nobilis*	Cyprinidae	0	2	0	0	0	0	2
Bigmouth Buffalo	*Ictiobus cyprinellus*	Catostomidae	18	19	1	0	1	0	39
Black Buffalo	*Ictiobus niger*	Catostomidae	2	0	0	0	1	0	3
Black Crappie	*Pomoxis nigromaculatus*	Centrarchidae	0	0	1	0	0	0	1
Brook Silverside	*Labidesthes sicculus*	Atherinopsidae	1	0	0	0	0	0	1
Channel Catfish	*Ictalurus punctatus*	Ictaluridae	0	3	0	0	0	1	4
Common Carp	*Cyprinus carpio*	Cyprinidae	0	0	0	0	2	2	4
Freshwater Drum	*Aplodinotus grunniens*	Scianenidae	0	1	0	0	0	0	1
Gizzard Shad	*Dorosoma cepedianum*	Clupeidae	5	14	0	1	0	1	21
Largemouth Bass	*Micropterus salmoides*	Centrarchidae	1	0	0	0	0	0	1
Quillback Carpsucker	*Carpiodes cyprinus*	Catostomidae	0	0	0	0	0	1	1
Red Shiner	*Cyprinella lutrensis*	Cyprinidae	0	0	6	0	0	0	6
Sand Shiner	*Notropis ludibundus*	Cyprinidae	2	0	0	0	4	0	6
Sauger	*Sander canadensis*	Percidae	0	0	1	0	0	0	1
Shorthead Redhorse	*Moxostoma macrolepidotum*	Catostomidae	0	1	2	0	0	1	4
Silver Carp	*Hypophthalmichthys molitrix*	Cyprinidae	1	0	1	0	1	0	3
Smallmouth Buffalo	*Ictiobus bubalus*	Catostomidae	10	10	10	6	10	5	51
Spotfin Shiner	*Cyprinella spiloptera*	Cyprinidae	2	0	2	0	2	0	6
Steelcolor Shiner	*Cyprinella whipplei*	Cyprinidae	2	0	7	0	8	0	17
White Bass	*Morone chrysops*	Moronidae	0	1	0	1	3	0	5
White Sucker	*Catostomus commersonii*	Catostomidae	2	0	0	0	0	0	2

Table 9. Catch per unit effort (CPUE) for fish species sampled using pulsed DC electrofishing upstream and downstream of the Sanitary District of Decatur in the Sangamon River on 29 November 2016.

Reach	Site	CPUE (fish per hour)
Upstream	1	92
	5	102
	8	62
		Upstream mean = 85.3
Downstream	9	16
	11	64
	12	22
		Downstream mean = 34

Table 10. Average total length (mm), standard length (mm), and caudal fin percent of total body length ± standard error for River Carpsucker, Smallmouth Buffalo, and Shorthead Redhorse from the Embarras and Sangamon Rivers. Significant differences in caudal percent were found between rivers for River Carpsucker and Smallmouth Buffalo.

River	Species	TL (mm)	SL (mm)	Caudal fin %
Sangamon				
	River Carpsucker	366.92 (4.98)	280.35 (4.60)	23.64 (0.58)
	Smallmouth Buffalo	474.58 (7.65)	361.77 (6.19)	23.81 (0.32)
	Shorthead Redhorse	386.12 (8.39)	313.48 (6.81)	18.80 (0.34)
Embarras				
	River Carpsucker	349.06 (4.48)	284.88 (3.83)	18.41 (0.24)
	Smallmouth Buffalo	434.25 (10.29)	355.70 (8.89)	18.23 (0.22)
	Shorthead Redhorse	365.72 (6.50)	298.19 (5.48)	18.49 (0.23)

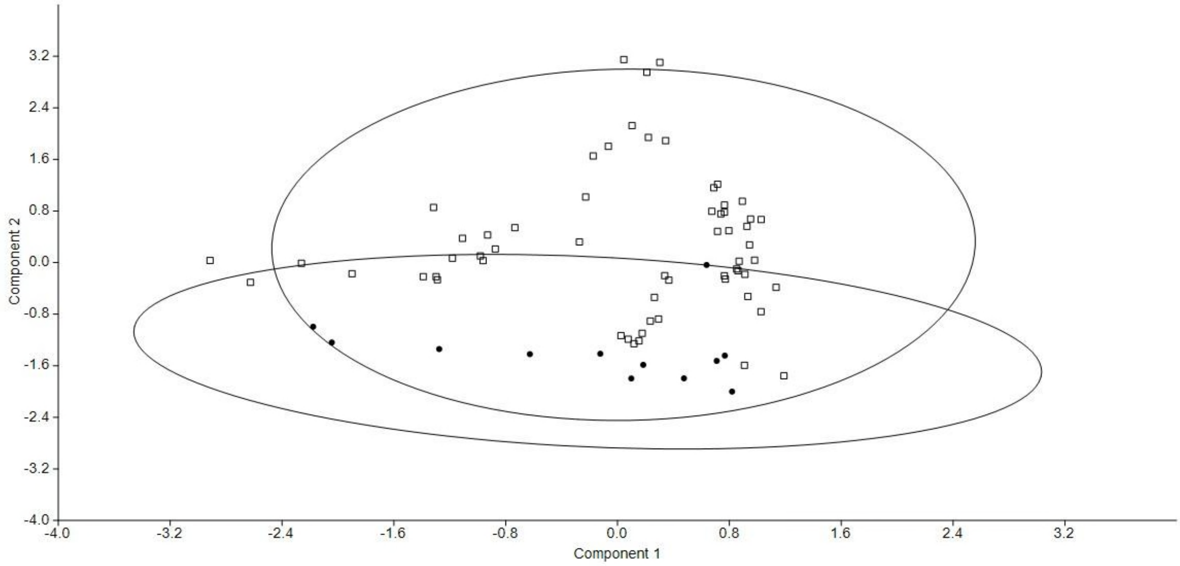


Figure 1. Principle components analysis of water quality data sampled during 2016-2017 from all mainstem water quality sites of the Sangamon River. Components 1 and 2 account for a total of 74.8% of the variation in the data. Variation in component 1 is largely due to temperature (r = 0.931) and dissolved oxygen (r = -0.273), whereas factor 2 is heavily influenced by total phosphate (r = 0.7199), total alkalinity (r = 0.473), and specific conductivity (r = 0.468). Samples collected from the downstream and upstream were significantly different (ANOSIM, Global R = 0.340, p < 0.001). Black circles represent the upstream reach, while open squares represent the downstream reach.

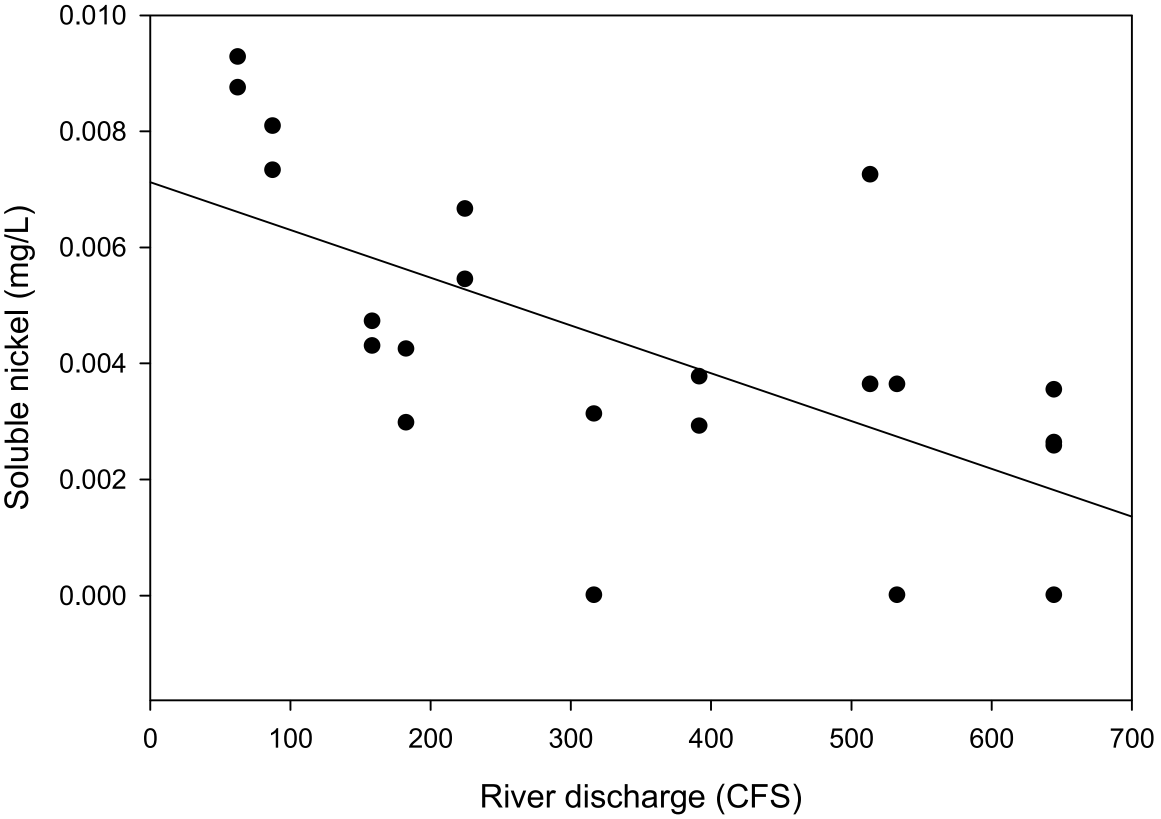


Figure 2. Scatter plot showing the relationship between nickel concentration and river discharge for sites 9 and 12. Points represent each sampling event at sites 9 and 12 from April 2016 – March 2017. River discharge data are from the Route 48 Bridge. Linear regression for river discharge versus nickel concentration showed a significant negative relationship (p= 0.001).

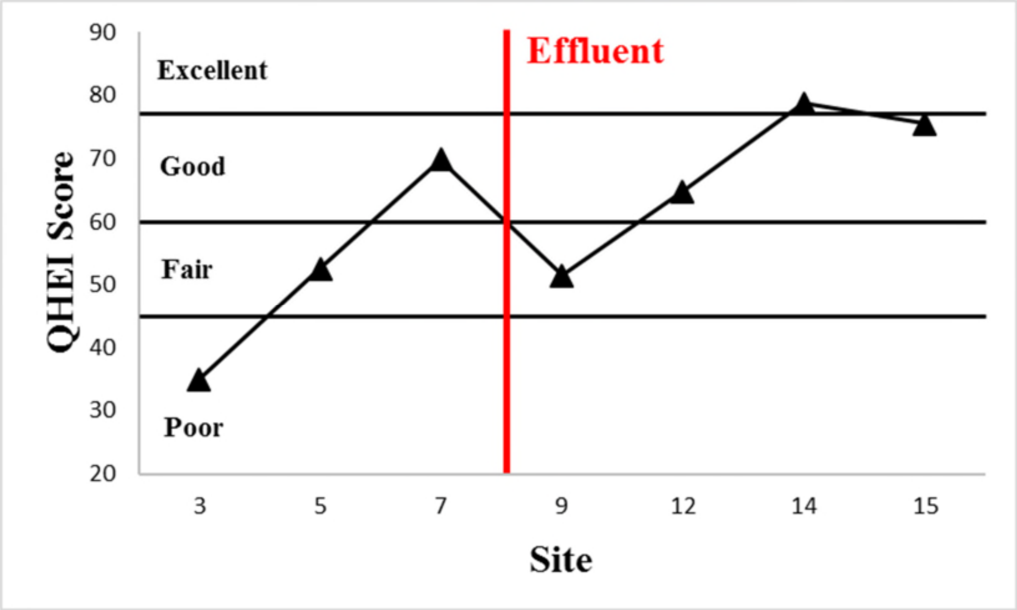
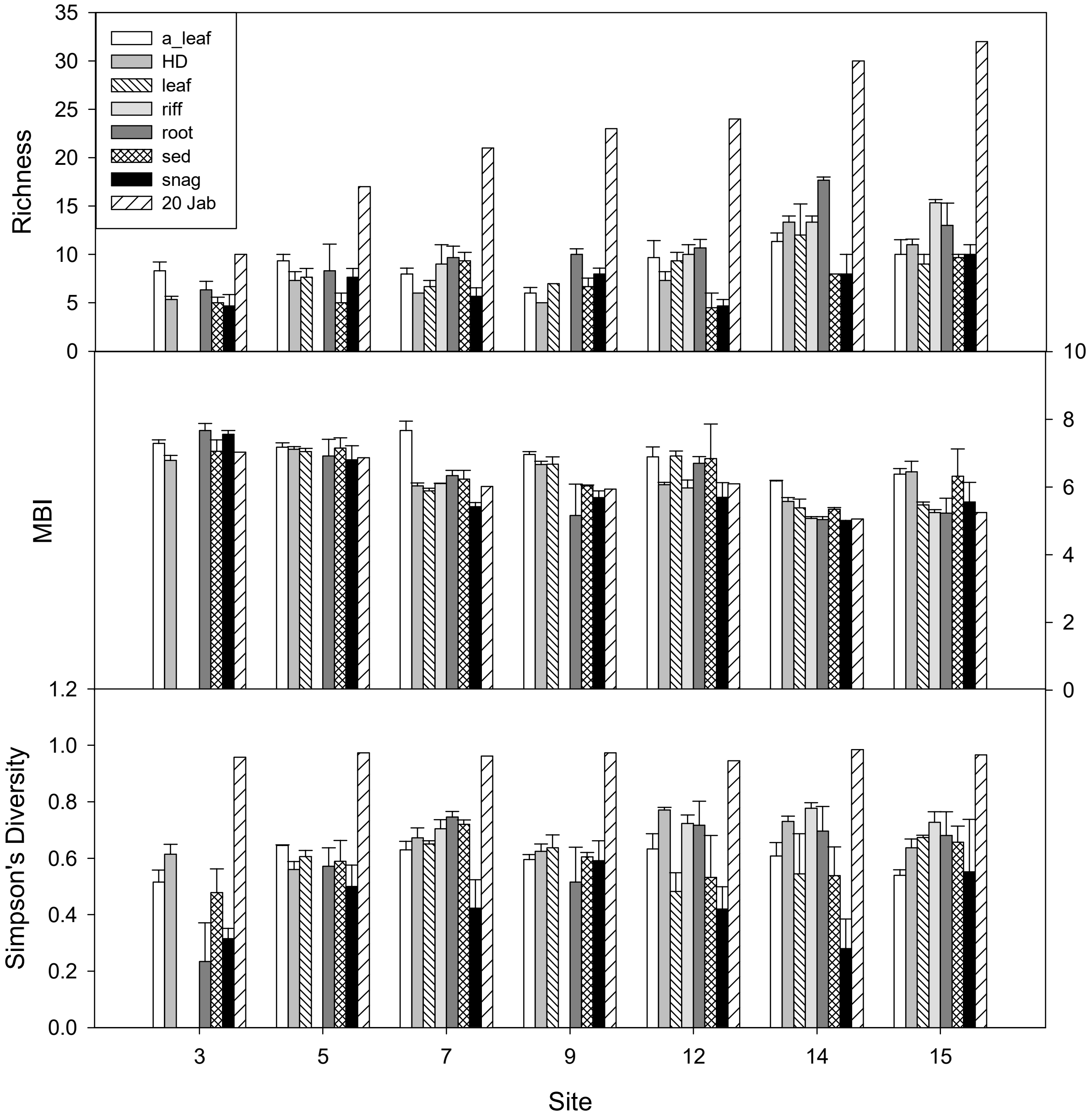


Figure 3. QHEI scores for each of the sites sampled with ranges indicating the quality (poor to excellent). Effluent is indicated by the red line. Sites listed in order from the most upstream site (3) to the most downstream site (15).



35
30
25
20
15
10
5
0
Richness
a_leaf
HD
leaf
riff
root
sed
snag
20 Jab
MBI
10
8
6
4
2
0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Simpson's Diversity
3
5
7
9
12
14
15
Site

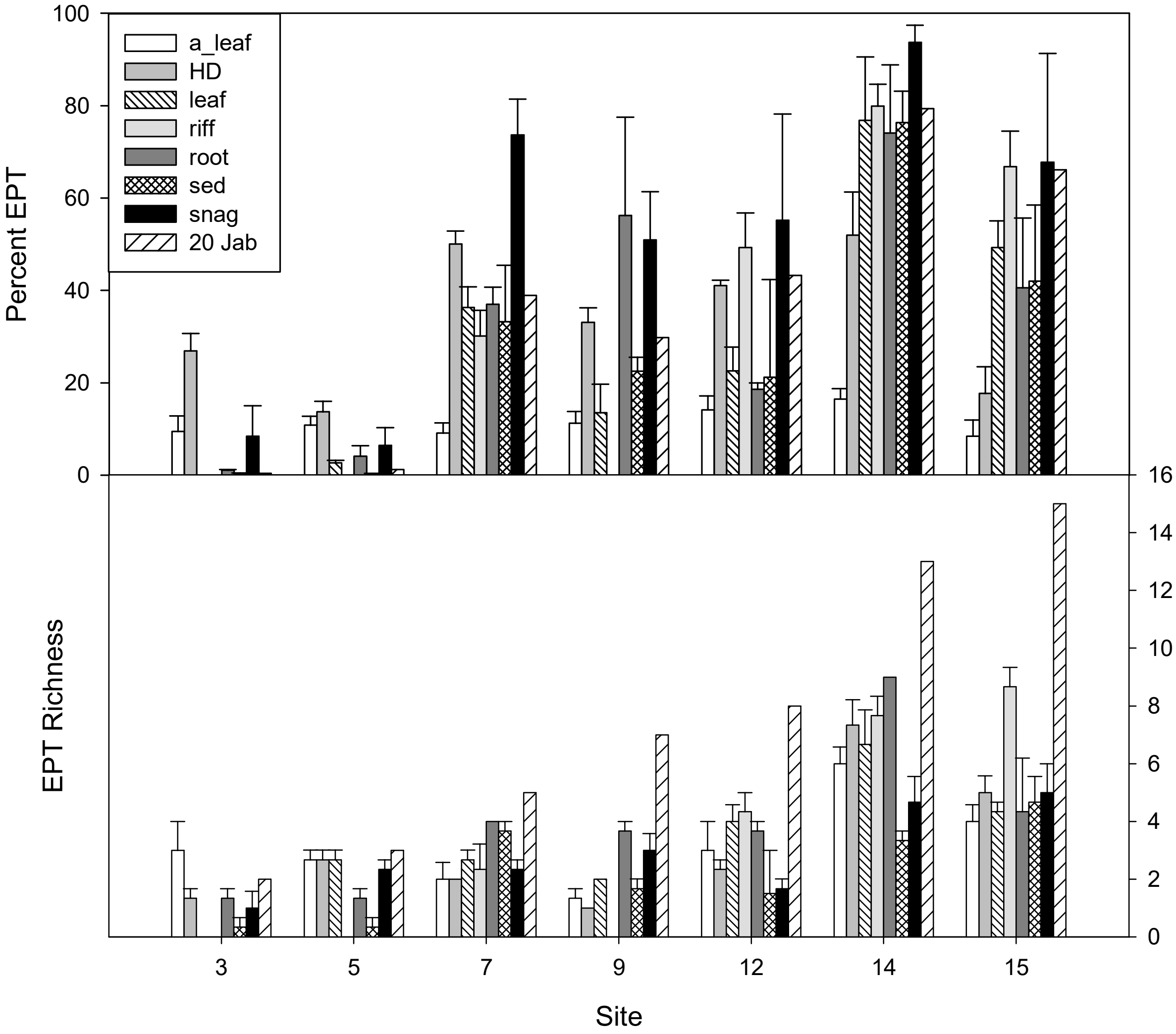


Figure 4. Graphs summarizing population indices of each natural habitat type, artificial habitats, and 20 jab samples for every site sampled. These indices include (top to bottom) richness, MBI, Simpsons Diversity, percent EPT, and EPT richness. P-values can be found in Tables 6 & 7.

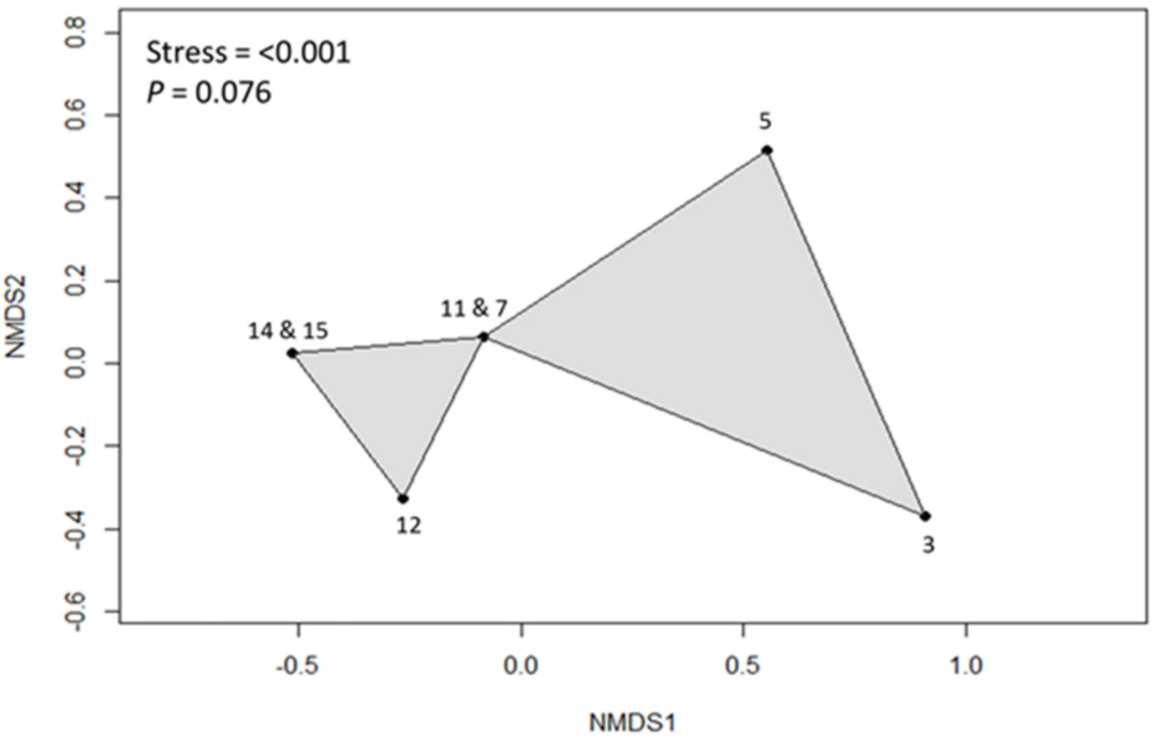


Figure 5. NMDS plot for macroinvertebrate communities based on 20 jab data for each site sampled.

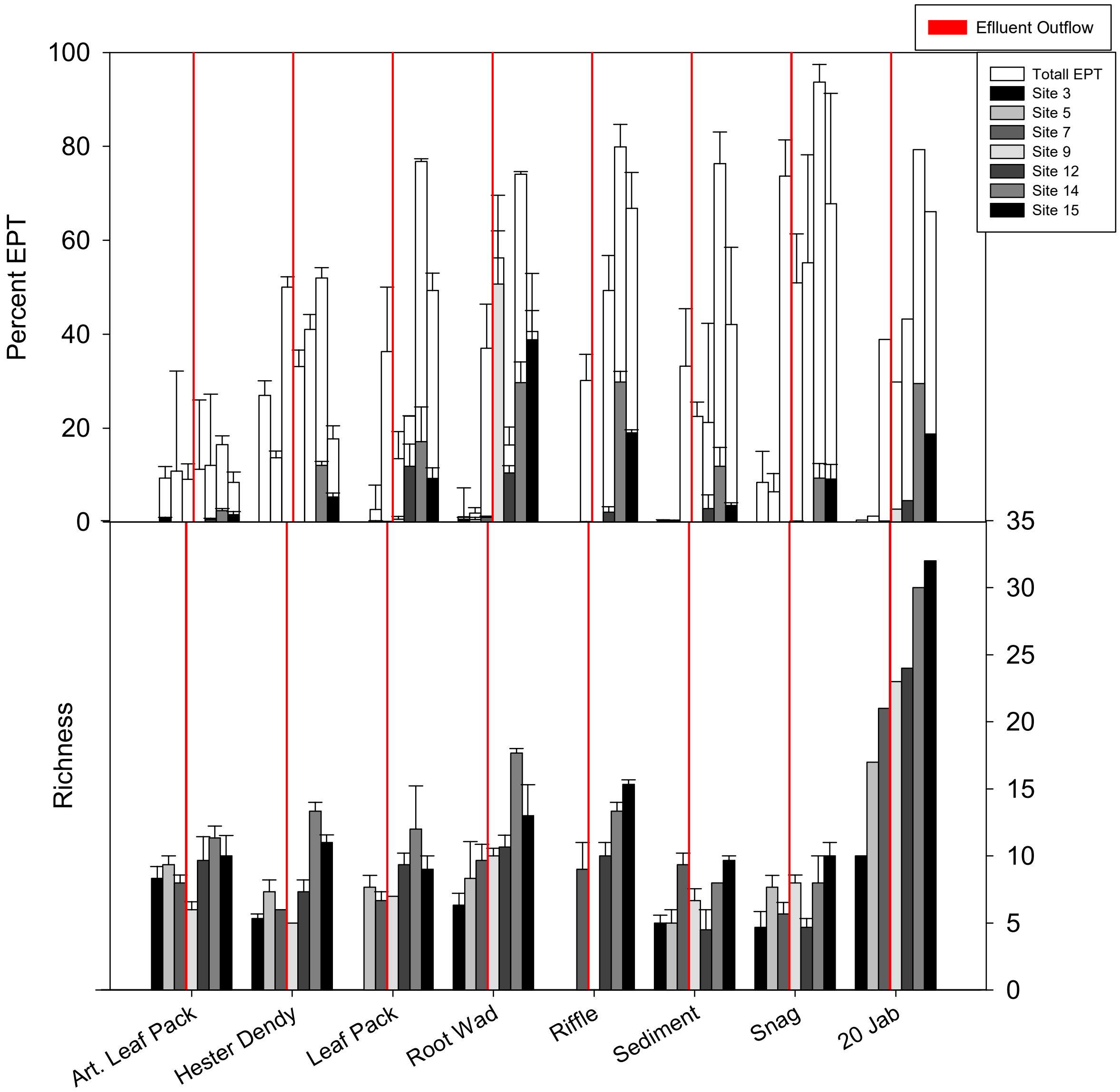


Figure 6. Graphs summarizing percent EPT and overall richness of each natural habitat type, artificial habitats, and 20 jab samples for every site sampled. Shaded bars for percent EPT represent taxa not belonging to the families Polycentropodidae and Hydropsychidae. White bars for percent EPT are the percent makeup of the members of the families Polycentropodidae and Hydropsychidae.

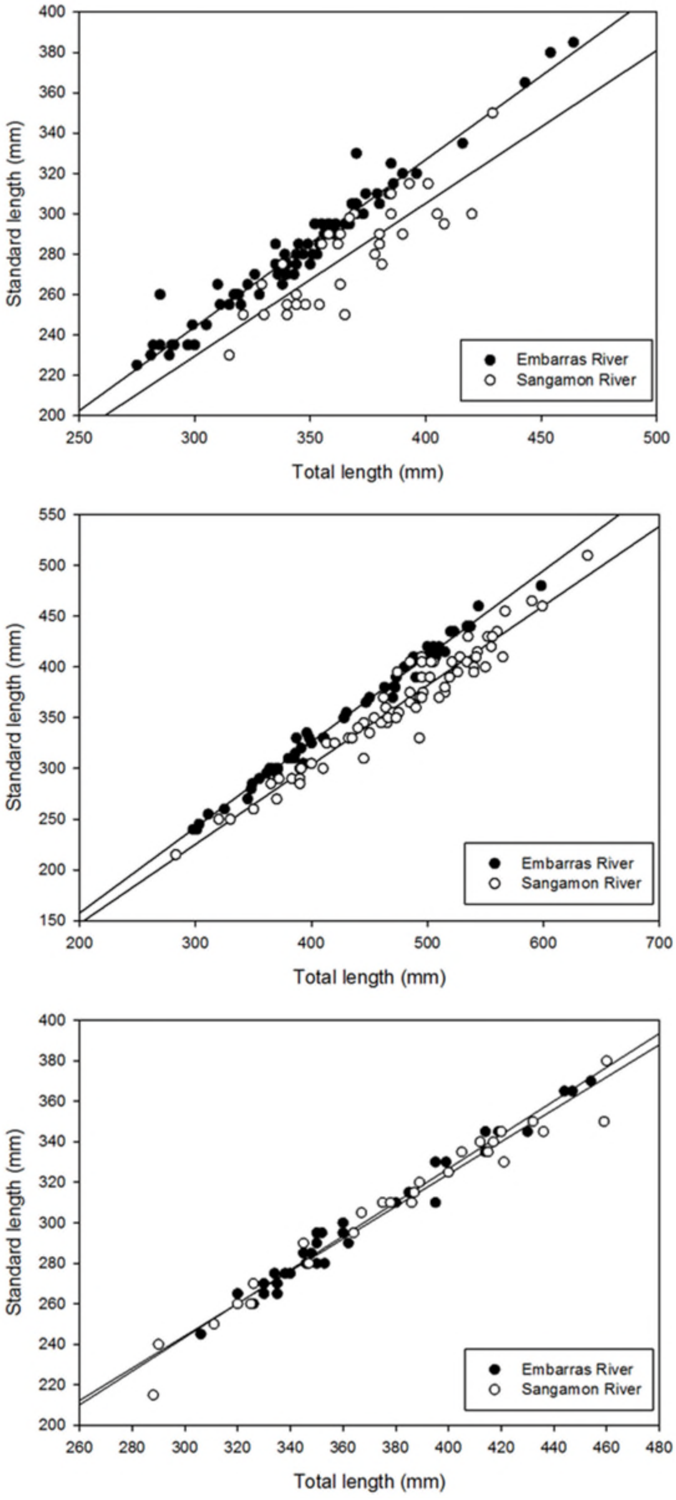


Figure 7. Total length (mm) versus standard length (mm) for River Carpsucker, Smallmouth Buffalo, and Shorthead Redhorse from the Embarras and Sangamon Rivers. Regression lines represent the relationship between total length and standard length.

APPENDIX

Sangamon River sites (Site # based on previous studies)

Site 1 – Lincoln Park CSO – above outfall
Site 3 – Lincoln Park CSO – below outfall
Site 4 – Oakland CSO (Lincoln Park) - above outfall
Site 5 – Oakland CSO (Lincoln Park) – below outfall
Site 6 – 7th Ward CSO (End Sunset Dr.) – above outfall
Site 7 – 7th Ward CSO (End Sunset Dr.) - below outfall
Site 8 – Main Treatment Plant (Off Main street) – upstream of main outfall
Site 9 – Main Treatment Plant (Off Main street) –downstream of main outfall
Site 11 – Sangamon River directly downstream of Stevens Creek
Site 12 – Bridge on Wyckles Road
Site 14 – Lincoln Trail Homestead State Park
Site 15 Mt. Auburn 2 miles north of Mt. Auburn
Site 16 Mechanicsburg Mechanicsburg Road Bridge

Monthly collections for water quality assessment were conducted at Sites 5, 9, 12, 14, 15, and 16. Macroinvertebrates were collected from Sites 3, 5, 7, 9, 12, 14 and 15.

Fish were collected from Sites 1, 5, 8, 9, 11, and 12.

Exhibit 33

Exhibit 33

Biotic assessment of water quality in a stretch of the Sangamon River receiving effluent from the Sanitary District of Decatur: Focusing on chemical assessment, macroinvertebrate assemblage, mussel assemblage, tiered-aquatic life use, and the sport fishery

Principal Investigators:

Robert E. Colombo, Ph.D.
Department of Biological Sciences
Eastern Illinois University
PHONE: (217) 581-3011
FAX: (217) 581-7141
recolombo@eiu.edu

Jeffrey R. Laursen, Ph.D.
Department of Biological Sciences
Eastern Illinois University
PHONE: (217) 581-3914
FAX: (217) 581-7141
jrlaursen@eiu.edu

Charles L. Pederson, Ph.D.
Department of Biological Sciences
Eastern Illinois University
PHONE: (217) 581-6239
FAX: (217) 581-7141
clpederson@eiu.edu

Research Assistants:

David Petry, dwpetry@eiu.edu
Clint Morgeson, cwmorgeson@eiu.edu

Submitted to:
Sanitary District of Decatur
501 Dipper Lane
Decatur, IL 62522

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

We sampled two treatment reaches of the Sangamon River for water quality, macroinvertebrate and fish assemblages, and Asian Carps. The two treatment reaches were 1) upstream of the Decatur Sanitary District main discharge (downstream of the Lake Decatur Dam), and 2) downstream of the main discharge. We sampled eleven sites monthly for water quality; seven sites located in the upstream reach, and four sites located downstream of the SDD. Seven sites were sampled during fall 2014 for macroinvertebrate and fish assemblages; four sites located in the upstream reach and three located in the downstream reach. Two reaches were sampled for Asian carp.

Water quality in the upstream and downstream reaches differed during periods when discharge, measured at the Route 48 Bridge, was below 200 cfs. Most macroinvertebrate indices showed no difference between the two reaches, except significantly higher percent EPT downstream of the SDD main effluent outfall. Further studies with a focus on specific microhabitats may discern finer differences between the reaches.

A total of nineteen fish species was sampled using seining methods during low water from the two treatment reaches of the Sangamon River. Because of high conductivity during low water, seines were implemented. Catch per unit effort was highest in the downstream reach (Site 11) and lowest in the upstream reach (Site 8). Steelcolor Shiner, Bluegill, and Spotfin Shiner made up over 63% of the total catch.

The Sangamon River was sampled for Asian Carps at least monthly from April – October 2014 in a reach downstream of the Lake Decatur Dam and a reach near Chandlerville. The Sangamon River supports a healthy population of adult (3-8 year old) Asian carp. Larval and

juvenile (0-2 year old) Asian Carp were unrepresented and were either nonexistent or were unable to be sampled.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Rivers and streams are impounded for a variety of reasons, including residential, commercial, and agricultural water supply; flood and debris control; and hydropower production (Kondolf 1997). However, impoundments may impact downstream aquatic systems and their surrounding terrestrial habitats. They can affect riverine systems by altering the flow regime, changing the sediment and nutrient loads, and modifying energy flow (Lignon *et al.* 1995). In addition, impoundments may lead to diminished water quality and availability, closures of fisheries, extirpation of species, and groundwater depletion for surrounding areas (Abramovitz 1996, Collier *et al.* 1996, Naiman *et al.* 1995). As a result of impoundments, downstream reaches may no longer be able to support native species, which will be reflected by reduced integrity of biotic communities (Naiman *et al.* 1995, NRC 1992).

A natural flow regime is critical for sustaining ecosystem integrity and native biodiversity in rivers (Poff *et al.* 1997). Depending on the use of the dam, it may have varying effects on downstream aquatic habitats. Impoundments used for urban water supplies lead to a reduction in flow rates downstream of the dam throughout the entire year (Finlayson *et al*. 1994), as well as increase daily and seasonal variability in flow regime (Finlayson *et al*. 1994, McMahon & Finlayson 2003). In addition, abiotic variables including temperature, dissolved oxygen, turbidity, pH, conductivity, and solids concentrations are altered in the downstream river system (Finlayson *et al.* 1994).

Along with stream and river impoundments, point source and non-point source pollution can have profound and lasting effects on the ecological integrity of the system. Non-point sources of pollution include agriculture, livestock grazing, and urbanization, and point source pollutions include sanitary discharge and industrial waste. In order to reduce point source pollution, the Water Quality Act of 1972 encouraged wastewater treatment plants to upgrade their systems. As a result, many communities were forced to build advanced tertiary water treatment facilities (Karr *et al.* 1985). Updated facilities still release high concentrations of nutrients into surrounding rivers. Carpenter and Waite (2000) documented that concentrations of phosphorus were highest in streams draining agricultural basins and at sites influenced by wastewater discharges, and Twichell et al. (2002) reported that sewage effluent inputs had elevated nitrate levels. The enhanced nutrient discharge can be expected to increase productivity within a river because primary productivity and detrital processing usually are limited by low ambient stream nutrient concentrations (Stockner and Shortreed 1978, Elwood et al. 1981, Winterbourn 1990).

Unlike impoundments and pollution, droughts are a natural phenomenon, but they can also severely affect aquatic ecosystems. Droughts can alter the lotic systems in ways harmful to biota, including loss of habitat, food resources, and stream connectivity (Lake 2003). The overall effect drought has on aquatic communities varies, and often depends on the availability of refugia and life history of the organisms (Humpheries and Baldwin 2003, Lake 2003). Macroinvertebrates, especially sensitive taxa such as stoneflies and caddisflies, can be temporarily decimated by drought conditions (Boulton 2003). The effects of a drought depend on many factors, including its severity, length, and the previous condition of the lotic system: specifically anthropogenic perturbations. Human disturbances such as impoundments can be exacerbated by drought conditions decreasing decrease the amount of dilution for pollution sources in lotic systems. This can lower the resilience of the aquatic ecosystem (Bond *et al.* 2008), potentially worsening their effects.

The Sangamon River

The Sangamon River flows for approximately 200 km in central Illinois, and its 14,000 km^2 watershed extends to 18 counties. Streams converging with the Sangamon River run through glacial and alluvial deposits, creating a low gradient stream with sand and gravel substrates. The Sangamon basin has experienced multiple point and non-point source impacts throughout the years. The Sangamon River watershed is dominated by agriculture and has the highest percentage of its land in crops of all major watersheds in the state (IDNR 2001). Major cities along the river include Bloomington, Decatur, and Springfield, and are home to more than 500,000 people. The Sangamon River immediately below Lake Decatur is influenced by impoundment, altered flow regime, and point source discharges.

Due to multiple anthropogenic influences, the biotic integrity of the Sangamon River is in constant flux. An intensive sampling program began in 1998-99 and continued through 2015 to document temporal and spatial heterogeneity of an 8.5 km urban reach of the Sangamon River. Sampling began directly below the Lake Decatur Dam and continued downstream to incorporate discharges from the Sanitary District of Decatur (SDD). These studies (Fischer & Pederson 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014) were intended to characterize stream habitat quality and assess impacts from ongoing reservoir and urban management by evaluating biotic integrity at various trophic levels in the context of the physical and chemical nature of the Sangamon River.

Original sampling locations were associated with operation of the Sanitary District of Decatur that were easily identified by landmarks within the city of Decatur, Illinois, USA. Sites were established in 1998 in conjunction with combined sewage overflow (CSO) facilities and the main treatment plant. Sites were located in the mainstem of the Sangamon River extending from directly below the Lake Decatur dam to the Lincoln Memorial Highway Bridge, located five miles southwest of Decatur. Sites 1, 3, 4, 5, 6, 7, and 8 extend from the dam to directly above the discharge of the main treatment plant in the upstream reach, and sites 9, 11, 12, and 14 extend from the main treatment discharge to a point approximately 8 river miles downstream near Lincoln Trail Homestead State Park.

Habitat Assessment and Water Chemistry

The Stream Habitat Assessment Procedure (SHAP), which assesses lotic habitat quality using features considered important to biotic integrity, was performed in 1998, 2001, and 2002. At each site, two individuals assessed metrics relating to substrate and instream cover, channel morphology and hydrology, and riparian and bank features to one of four habitat quality types, following guidelines established by the Illinois Environmental Protection Agency (1994). The average total score of the 15 metrics form the basis of an overall habitat quality rating for the stream reach under consideration. The SHAP was replaced with the Qualitative Habitat Evaluation Index (QHEI) (Rankin 1989) starting in 2010 and was repeated in 2012 and 2013. It is as a more rigorous measure of physical habitat that also incorporates objective invertebrate sampling.

This overall physical structure based on substrate characteristics, channel morphology, and bank features provides a basis for the potential of the study reach to support diverse aquatic life. A routine assessment of characteristic water quality variables and biotic assessments in these sites allow us to evaluate whether the stream is, in fact, reaching this potential. Aquatic organisms often exist in narrow ranges of tolerance for water chemistry. We have compared physical and water chemistry parameters of the Sangamon River sites from 2002-2015.

Tiered Aquatic Life Use

We sought to assess the water quality, as well as the macroinvertebrate, fish, and Unionid mussel communities of the Sangamon River near Decatur, Illinois. We sampled the communities in two treatment reaches; one above and one below the Decatur Sanitary District main effluent. Although all of these metrics individually provide some measure of habitat, the combination of all data will provide a more broad analysis of multiple uses as it pertains to the Tiered Aquatic Life Use (TALU).

The Tiered Aquatic Life Use is a measure of the quality of habitat. It includes both biotic and abiotic assessment of a given resource to determine how closely habitats compare to potential. The goal is to identify any factors affecting this ratio as targets for remediation. The TALU includes not only historic ecological indices of multiple trophic levels of biota, but economic and recreational value of an aquatic system as well. For example, although sportfish make up a small portion of the fish assemblage they almost always have the greatest economic and recreational value. As top predators, they may reflect changes in lower trophic levels, but many are not especially sensitive to water quality. In contrast, Unionid mussels have shown sensitivity to various assaults on lotic systems. Mussels can be affected by substrate type and flow (Harman 1972; Strayer 1983; Vaughn 1997; Watters 1999), and can be harmed by excessive concentrations of heavy metals, phosphorus, and nitrogen (Beckvar *et al.* 2000; Jacobson et al 1997; Mummert et al 2003; Wang et al 2007). As such, the U.S. Environmental Protection Agency proposed using mussel communities in setting ammonia standards (Great Lakes Environmental Center 2005). Based on this information it is important to include these specific communities in assessment of aquatic systems.

Population Demographics of Invasive Asian Carps

Since the invasion of Silver (*Hypophthalmichthys molitrix*) and Bighead (*H. nobilis*) Carps in North America, collectively referred to as Asian Carp, they have established themselves in high abundances within the Mississippi River system. The Illinois River, a principle tributary of the Mississippi River, connects the Great Lakes to the Mississippi River system. Asian Carp establishment in the Great Lakes has the potential to cause a massive negative impact to the ecological and economical systems of the Great Lakes.

The purpose of this study is to evaluate Asian Carp populations in tributaries of the Illinois River, determining population demographics and documenting potential spawning activity. The Sangamon River is included in this study, as is the Salt Creek, Spoon, Mackinaw, and Kankakee Rivers. Asian Carp have been shown to spawn in rivers with long stretches of flowing water, of which requirements are not fulfilled in the Great Lakes. These conditions do exist within the tributaries of the Great Lakes and by sampling these tributaries, we expect to look at a surrogate of areas to target management should Asian Carp become established.

Thus beginning in 2013, Eastern Illinois University began collecting data to analyze population dynamics of invasive Asian Carp in tributaries of the Illinois River. This report includes a summary of the current progress of this study in the Sangamon River.

METHODS

Water Data Collection and Chemistry Determination

We collected water quality data monthly from April 2014 to March 2015. Sampling began at the Lake Decatur Dam and proceeded downstream. In the field, we used a YSI Professional Plus handheld meter to measure dissolved oxygen, temperature, and specific conductivity, and pH. In May of 2013 we added a chloride probe to the meter and began taking measurements of chloride concentrations every month afterward. Water samples were collected just below the surface, returned to the lab on ice, and analyzed within accepted time limits. All analyses followed the Standard Methods for Examination of Water and Wastewater (APHA, 1995).

Suspended and total solids were determined by drying residue collected on standard glass fiber filters (suspended solids) and unfiltered samples (total solids) at 103-105 ^{o}C. We analyzed volatile and suspended solids by weight loss upon ignition at 550 ^{o}C. Total oxidized nitrogen (NO_2-N + NO_3-N) was determined using the cadmium reduction method, and ammonia nitrogen was determined with the phenate method. We used the ascorbic acid method to determine total phosphorus (following persulfate digestion) and soluble reactive phosphate (following filtration). A Thermo Scientific Evolution 300 UV-VIS spectrophotometer was used for all colorimetric nutrient analyses. Hardness and alkalinity were measured using titration to colorimetric endpoint methods. We considered quality control procedures during all analyses, including but not limited to parallel analyses of laboratory standards.

We calculated and report the averages of each variable for the upstream and downstream reaches. In order to determine overall differences between reaches, principle components analysis (PCA) was conducted for 15 variables after individually log transforming and normalizing the data. Variables that were highly correlated to another and thus redundant were eliminated from the analysis. All analyses were performed using Primer 6.1.14 (Clarke and Warwick 2001). Correlation analysis was performed to determine the relationship among measured and derived variables.

Assessment of Macroinvertebrate and Freshwater Mussel Communities

Macroinvertebrates were sampled during fall 2014 using a modification of Illinois RiverWatch protocol. We sampled four sites below the dam and above the effluent and three sites within 15 miles downstream of the effluent (Appendix 1). At each site, we sampled the "best" four habitats present. Illinois RiverWatch ranks "best" habitats based on their potential to host the greatest diversity of macroinvertebrates as follows: riffle, leaf pack, snag, undercut bank, and sediment. This is in contrast to previous 20 jab sampling efforts which sampled all habitat proportionally according relative abundance of each habitat type based on QHEI.

We preserved the macroinvertebrates in 70% ethanol and transported them to the EIU Fisheries and Aquatic Research Lab for identification and enumeration. In the lab, we subsampled macroinvertebrates from each site using a thirty grid tray. Grids were selected at random until a target of at least 300 macroinvertebrates were picked with a minimum of three grids (10% of the sample) selected, plus any large or rare taxa. All individuals were identified to taxonomic levels required by Illinois RiverWatch (typically family-level). Voucher specimens were catalogued into the EIU invertebrate collection.

We assessed the relative abundance, taxonomic richness, Simpson's diversity (D), percent Ephemeroptera, Pleucoptera, and Trichoptera (EPT) taxa, and macroinvertebrate index of biotic integrity (MIBI) based on taxon-specific environmental sensitivity values using the Illinois RiverWatch protocol. The Simpson's diversity (D) was calculated using the formula:

$$D = \frac{1}{\Sigma p_i^2}$$

- where p_i = is the proportion of the total number of individuals comprised by species *i.*

We performed an ANOVA to assess differences between upstream and downstream sites. Sites were plotted using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity matrices. Data were square root transformed and standardized by abundance and site. Analyses were performed using R (version 3.1.2; R Foundation for Statistical Computing 2014) and required the vegan package (version 2.2-1; Oksanen et al. 2013).

Mussel assemblages were not sampled in 2014 due lack of mussels found in previous years.

Assessment of Fish Community

We also sampled fish communities at four sites upstream of SDD (Sites 3, 5, 7, and 8), and three sites downstream of SDD (Sites 11, 12 and 14) on 22 September 2014 using the pull seine method. At each site, we completed two seine pulls which required one person to hold one end of the seine near shore, staying in place, while a second person pulled the seine out into the middle of the river and continued upstream, wrapping around to meet the person near shore. An estimate of relative density was calculated as catch per unit effort (CPUE) as number of fish captured per seine pull. Fish were immediately euthanized in formalin and were taken back to the laboratory for identification.

Population Dynamics of Invasive Asian Carps

Study Area

Beginning in 2013, four tributaries of the Illinois River were sampled each year. Each river was sampled at two sites, one at a downstream location near the mouth and one at an upstream location, chosen to maximize distance between sites while considering accessibility, impoundments, and occurrence of Asian Carps. The Sangamon River was sampled downstream near the town of Chandlerville (SG3) and upstream below the dam in Decatur (SG1).

Larval Fish Sampling

Larval fish sampling of each tributary river occurred at least monthly from April-October at the mouth/downstream sites when accessible. In the spring and summer months, peak spawning times for Asian carp (Kolar et al. 2005), larval fish sampling was conducted weekly to increase chances of encounter. A 500 µm mesh conical cylindrical ichthyoplankton net measuring 0.5 m x 3 m mounted to a rigid frame at the front of a boat was pushed against the current for five minutes at each site. A flow meter was attached in the center of the mouth of the net to estimate average velocity and volume of water sampled. The net was operated just under the surface of the water and sampled the top half meter of the water column for suspended and pelagic eggs and larvae. Three five-minute ichthyoplankton samples were taken at each downstream site, one near each bank and a main channel sample, equidistant between each bank.

All larval samples were fixed in 95% ethanol in the field. Fixed samples were then returned to the laboratory for enumeration and subsequent identification based on meristic, morphometric, and composite characteristics. Following identification, each specimen was measured for total length (mm). Catch per volume sampled, or density, was calculated for each site in fish per cubic meter of water sampled.

Adult Fish Sampling

Asian Carp were sampled using boat pulsed-DC electrofishing (60 Hz, 25% duty cycle) monthly at fixed sites, both upstream and downstream, from April – October 2014. A single fifteen minute transect using a modified Long Term Resource Monitoring Program (LTRMP) protocol (Gutreuter et al. 1995) was employed to capture fish; only Asian Carp were collected. Power output was maximized according to local specific conductivity measurements with a Wisconsin ETS electrofishing box. Each collected fish was measured (mm), weighed (g), sexed and gonad development staged via direct gonadal observation (Li and Mathias 1994), gonad weight taken (g), assigned a unique identification number, and had a postcleithrum bone removed for aging estimation. Postcleithra were returned to the lab to be cleaned and sectioned sequentially in the transverse plane using an ISOMET® low speed saw to 0.5-1 mm thickness (Johal et al. 2000). Three sections were then mounted to glass slides for imaging using a camera-equipped Leica® stereoscopic microscope with a contrast background. Images were aged by two independent readers, disagreements were subject to discussion between the readers. Ages that could not be agreed upon were excluded from further analysis.

Water Quality

Water temperature (°C), dissolved oxygen (mg/L), conductivity (μ*s*), flow (m/s), secchi depth (cm), river stage (ft), and discharge (ft^3/s) was collected for each sampling event.

Data Analysis

Larval fish densities were calculated as number of fish per cubic meter of water sampled. Length frequency and age frequency histograms were created for adult fish in the Sangamon River. Additionally, relative abundance (individuals sampled/amount of effort) and sex ratios (males:females) within the sites sampled were also calculated.

RESULTS

Water Data Collection and Chemistry Determination

A total of 19 water quality variables were determined for eleven sites along the Sangamon River (Table 1). Principle Components Analysis extracted five factors that explained 76.8% of the total variation in water quality of the Sangamon River during the sampling period. Discrete sampling events (site, date) cluster on the basis of discharge and stream reach (Figure 1). Variables included in the analysis were temperature, dissolved oxygen, pH, conductivity, hardness, total alkalinity, total oxidized nitrogen, ammonia, total phosphorus, fixed suspended solids, volatile suspended solids, fixed dissolved solids, and volatile dissolved solids. PCA extracted five factors which account for a total of 76.8 % of the variation in the data. Variation in factor 1 is largely due to conductivity ($r = 0.420$), hardness ($r = 0.395$), temperature ($r = -0.392$), and fixed dissolved solids ($r = 0.360$), whereas factor 2 is heavily influenced by total phosphorus ($r = 0.562$). Samples collected from the downstream and upstream were significantly different (ANOSIM, Global $R = 0.246$, $p < 0.001$) with more profound differences observed during periods of low reservoir discharge. Overall differences between upstream and downstream reaches continue to be influenced largely by total and/or soluble phosphate from the SDD treatment facility.

Assessment of Macroinvertebrate Community

A total of 25 different taxa was identified from the eight sites sampled (Table 2). There was no significant difference between the 2 reaches for estimated relative abundance ($F_{1,5} = 2.486$, $p = 0.176$) , total taxa richness ($F_{1,5} = 1.000$, $p = 0.363$), EPT richness ($F_{1,5} = 0.765$, $p = 0.422$), Simpson's Diversity ($F_{1,5} = 0.397$, $p = 0.556$), or MBI ($F_{1,5} = 3.964$, $p = 0.103$) (Figure 2).

The percent EPT, however, was significantly higher downstream of SDD main outfall ($F_{1,5}$ = 21.69, *p* = 0.006) (Figure 2).

MIBI scores ranged between 5.22 ("poor") to 6.68 ("very poor"). Midges were much more abundant in the upstream reach while Hydropsychid caddisflies were much more abundant in the downstream reach. Stoneflies (one of the most intolerant taxa) were previously not sampled using proportional habitat sampling, but were collected in both reaches when sampling best habitats. Taxa unique to the upstream reach included sowbugs, dragonflies, "other" caddisflies, biting midges, right-handed snails and planorbid snails. Taxa unique to the downstream reach include broadwinged damselflies, swimming mayflies, and riffle beetles. Multidimensional scaling revealed differences in assemblage structure between the reaches and explained about 78% of the variation ($p = 0.028$; $R^2 = 0.78214$; Figure 3).

Assessment of Fish Community

Seine pulls were conducted at a total of 7 sites: 4 upstream of SDD and 3 downstream of the Sanitary District of Decatur. We sampled a total of 341 individuals from 19 species (Table 3). The three most dominant species sampled were Steelcolor Shiner (*Cyprinella whipplei*), Bluegill (*Lepomis macrochirus*), and Spotfin Siner (*Cyprinella spiloptera*), and comprised over 63% of the total catch. The sportfish community in the Sangamon River was comprised of sunfishes (*Lepomis* sp.), White Crappie (*Pomoxis annularis*), and Largemouth Bass (*Micropterus salmoides*) (Table 3). The non-sportfish community was dominated by Steelcolor Shiner (*Cyprinella whipplei*), Spotfin Shiner (*Cyprinella spiloptera*), Sand Shiner (*Notropis ludibendus*), and Cyprinidae. (Table 3).

Relative density as estimated by catch per unit effort (CPUE) as fish per seine pull was highest in the downstream reach at Site 11 and lowest in the upstream reach at Site 8 (Table 4).

Population Dynamics of Invasive Asian Carps

In 2014, a total of 220 adult Asian Carp was collected in the Sangamon River (Table 5). A total of 210 minutes of effort was expended in 2014, with 95 minutes of larval effort and 210 minutes of electrofishing effort. No larval Asian Carp were collected but 189 larval fish from 8 families were sampled in the Sangamon River in 2014 at the downstream site (Table 6). Because of the low catch rate of Bighead Carp, they will be combined for analyses with Silver Carp. Densities of both fish and eggs were highest in June (Figure 4). Total relative abundance of adult Asian Carp in the Sangamon River was 63 ± SE 16 ind/hr. Asian Carp ranged in size from 500-805 mm (mean 594.98 ± SE 3.78 mm), weight from 1250-5000 g (mean 2323 ± SE 47.52), and age from 3-8 yr (mean 5.37 ± SE 0.06 yr) (Figures 5 and 6). Asian Carp lengths by site type (upstream, downstream) were upstream mean 608.53 ± SE 6.36 mm and downstream mean 582.61 ± SE 4.00 mm (Figure 5). Mean age by site type were upstream mean 5.31 ± SE 0.11 yrs and downstream mean 5.43 ± SE 0.08 (Figure 6). The Sangamon River displayed a nearly equal sex ratio, with 101 females and 119 males.

DISCUSSION

The primary differences between the upstream and downstream reaches are likely attributable to metrics related to reservoir discharge and inputs from the SDD main discharge. Outflow from Lake Decatur is the primary input to the upstream reach, which is compromised as a result of management to maintain reservoir levels by eliminating outflow. The discharge from the main treatment plant of the SDD alters instream water chemistry, especially during periods of low reservoir discharge. Consistent flow downstream of the SDD's main outfall during low discharge periods may help maintain physical habitat quality while the upstream reach becomes disconnected pools.

The macroinvertebrate community above the effluent was heavily dominated by aquatic midges. Midges are common in organic rich habitats and are often the most abundant taxa in these habitats (Rabeni and Wang 2001). Most metrics commonly used to describe water quality (e.g., taxa richness, MBI) were not significantly different between reaches sampled, with the exception of percent EPT which was significantly higher in the downstream reach. Previously absent from samples, Stoneflies were collected by sampling the best habitats and were found in both reaches. Increased sampling effort in the best habitats resulted in the addition of these very intolerant individuals. Quality habitat availability is likely the primary factor influencing differences in macroinvertebrate assemblages, and when present, is able to serve as refugia for sensitive taxa.

For this reason, we propose that future sampling focuses on macroinvertebrate assemblages of microhabitats (e.g. riffle, leaf pack, snag). This change will allow us to determine the influence of habitat on a smaller scale. Potential habitat restoration should attempt to maximize the most productive microhabitats to support the largest diversity of macroinvertebrate assemblages.

The diversity of fish species was comparable to other Midwestern streams (Colombo unpublished data), with Steelcolor Shiner and Spotfin Shiners being the most numerically abundant non-game species and Bluegill being the most abundant sportfish species. Future sampling using multiple gears will allow a more accurate representation of the species composition; these data will be used to determine the economic value and best management strategy for the fishery in the Sangamon River.

Our data show that Asian Carp in the Sangamon River in 2014 consist of an adult population 3-8 years of age. Larval and juvenile fish age 0-2 are unrepresented in the data, showing that these individuals were either ineffectively sampled by our gears, or do not exist in the Sangamon River in detectable quantities. Length frequency histograms show a broader distribution of size in the upstream site compared to the downstream site, suggesting either greater growth or longer lived, larger individuals occurring here compared to the downstream location. This is supported in the age data, with relatively few 3-4 year old fish and higher numbers of 5-7 year old fish in the upstream location, which would presumably be of larger body size. Within the entire river, we saw a concentration of 5-7 year old fish, and 3 year old fish were sampled exclusively in the upstream location. We would expect to find younger fish nearer to their spawning locations, but more information is needed to discern the recruitment source of these 3 year old individuals. Although we did not find direct evidence of Asian Carp spawning activity, it appears that Asian Carp exist in the Sangamon River in an abundant, healthy adult population.

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TABLES AND FIGURES

Table 1. Measured water quality variables for eleven Sangamon River sites associated with the Sanitary District of Decatur. Variables below the detection limit are indicated as 0.00. Missing data are indicated by blank cells.

Date	Site	DO mg L^{-1}	Temp (°C)	pH	Spec. Cond. mS cm^{-1}	Hard. mg L^{-1}	Total Alk. mg L^{-1}	NO_2/ NO_3 mg L^{-1}	NH_4 mg L^{-1}	PO_4 total mg L^{-1}	PO_4 SRP mg L^{-1}	TSS mg L^{-1}	FSS mg L^{-1}	VSS mg L^{-1}	TDS mg L^{-1}	FDS mg L^{-1}	VDS mg L^{-1}	TS mg L^{-1}	TFS mg L^{-1}	TVS mg L^{-1}	Gage Ht (ft)	Discharge (cfs)
04/28/14	1	13.1	16.9	7.6	412	204.4	153.6	4.17	0.03	0.17	0.00										5.71	585
05/19/14	1	12.7	16.8	7.1	447	233.6	195.4	2.36	0.04	0.12	0.04	21.6	12.0	9.6	370.4	216.0	154.4	392.0	228.0	164.0	8.8	1600
06/16/14	1	10.5	22.8	7.6	519	240.9	223.4	7.11	0.07	0.18	0.12	23.1	17.3	5.8	400.9	214.7	186.2	424.0	232.0	192.0	4.87	389
07/14/14	1	12.0	26.0	7.6	513	251.9	195.4	7.58	0.19	0.25	0.09	23.5	17.5	6.0	339.2	189.2	150.0	362.7	206.7	156.0	11.5	2890
08/11/14	1	7.7	24.1	8.1	414	251.9	363.0	4.56	0.25	0.21	0.12										3.29	154
09/15/14	1	8.7	18.9	8.3	458	332.2	125.6	2.15	0.39	0.29	0.19	16.4	11.6	4.8	328.9	145.7	183.2	345.3	157.3	188.0	6.42	823
10/13/14	1	14.3	14.2	8.3	600	492.8	279.2	5.26	0.00	0.19	0.12	9.6	6.4	3.2	385.1	133.6	251.5	394.7	140.0	254.7	5.14	492
11/10/14	1	11.4	8.3	7.9	793	434.4	223.4	4.77	0.21	0.11	0.08	10.4	6.4	4.0	402.9	240.3	162.7	413.3	246.7	166.7	3.22	146
12/08/14	1	4.7	2.2	7.6	797	584.0	209.4	4.44	0.07	0.17	0.02	8.8	4.8	4.0	415.2	255.2	160.0	424.0	260.0	164.0	5.2	504
01/20/14	1	0.3	2.9	7.8	871	361.4	223.4	6.62	0.03	0.19	0.15	12.8	10.4	2.4	501.9	268.3	233.6	514.7	278.7	236.0	4.26	379
02/17/15	1	17.1	0.9	7.8	800	292.0	209.4	7.71	0.01	0.13	0.06	7.2	4.8	2.4	474.1	396.5	77.6	481.3	401.3	80.0	3.96	307
03/17/15	1	10.9	2.8	7.7	831	372.3	209.4	0.80	0.01	0.03	0.00	3.2	2.4	0.8	418.1	260.3	157.9	421.3	262.7	158.7	6.91	1240
04/28/14	3	11.9	17.0	8.4	413	193.5	251.3	4.21	0.08	0.16	0.04										5.71	585
05/19/14	3	11.9	16.8	7.9	447	222.7	181.5	3.69	0.04	0.15	0.07	22.4	12.8	9.6	365.6	217.9	147.7	388.0	230.7	157.3	8.8	1600
06/16/14	3	10.7	22.8	7.8	524	233.6	167.5	7.55	0.03	0.19	0.10	24.0	19.0	5.0	373.3	214.3	159.0	397.3	233.3	164.0	4.87	389
07/14/14	3	9.7	26.1	8.2	510	281.1	195.4	4.99	0.20	0.25	0.15	24.0	17.5	6.5	333.3	195.8	137.5	357.3	213.3	144.0	11.5	2890
08/11/14	3	7.2	24.2	8.3	414	261.0	153.6	2.36	0.11	0.18	0.06										3.29	154
09/15/14	3	8.8	18.9	8.4	458	273.8	153.6	0.90	0.27	0.28	0.12	16.4	11.2	5.2	316.9	167.5	149.5	333.3	178.7	154.7	6.42	823
10/13/14	3	12.3	14.3	8.1	598	492.8	251.3	4.88	0.00	0.21	0.12				380.3	115.2	265.1	380.3	115.2	265.1	5.14	492
11/10/14	3	11.9	8.3	8.0	788	335.8	209.4	5.37	0.14	0.13	0.02	10.0	5.6	4.4	408.7	231.7	176.9	418.7	237.3	181.3	3.22	146
12/08/14	3	0.6	2.2	7.7	799	365.0	279.2	4.66	0.08	0.15	0.04	8.8	4.0	4.8	403.2	250.7	152.5	412.0	254.7	157.3	5.2	504
01/20/14	3	0.3	2.9	7.9	869	361.4	181.5	7.04	0.01	0.17	0.14	9.6	7.2	2.4	509.1	276.8	232.3	518.7	284.0	234.7	4.26	379
02/17/15	3	16.7	1.0	7.9	806	401.5	558.4	8.24	0.02	0.11	0.07	7.2	4.4	2.8	476.8	395.6	81.2	484.0	400.0	84.0	3.96	307
03/17/15	3	10.4	2.8	7.6	831	321.2	181.5	0.73	0.03	0.00	0.01	4.4	3.2	1.2	414.3	260.8	153.5	418.7	264.0	154.7	6.91	1240

04/28/14	4	11.2	17.1	8.6	412	197.1	97.7	3.53	0.02	0.18	0.00										5.71	585
05/19/14	4	10.6	16.8	8.3	447	248.2	181.5	4.36	0.04	0.18	0.03	22.8	13.6	9.2	369.2	206.4	162.8	392.0	220.0	172.0	8.8	1600
06/16/14	4	11.2	22.7	8.0	550	262.8	181.5	9.40	0.03	0.20	0.09	31.5	25.0	6.5	389.8	203.0	186.8	421.3	228.0	193.3	4.87	389
07/14/14	4	8.6	26.1	8.4	508	262.8	223.4	4.99	0.21	0.20	0.09	27.5	21.5	6.0	333.8	207.8	126.0	361.3	229.3	132.0	11.5	2890
08/11/14	4	8.1	24.1	8.3	427	255.5	195.4	2.50	0.04	0.12	0.10										3.29	154
09/15/14	4	8.4	18.9	8.5	457	266.5	153.6	0.74	0.21	0.27	0.26	17.6	12.4	5.2	327.7	155.6	172.1	345.3	168.0	177.3	6.42	823
10/13/14	4	11.1	14.3	8.1	603	365.0	237.3	6.35	0.00	0.22	0.09	14.0	10.4	3.6	382.0	144.3	237.7	396.0	154.7	241.3	5.14	492
11/10/14	4	11.0	8.3	8.2	801	350.4	209.4	5.83	0.00	0.06	0.06	9.2	4.4	4.8	416.1	207.6	208.5	425.3	212.0	213.3	3.22	146
12/08/14	4	0.5	2.2	8.0	803	328.5	418.8	4.31	0.00	0.11	0.08	9.6	4.4	5.2	435.7	280.9	154.8	445.3	285.3	160.0	5.2	504
01/20/14	4	0.2	2.9	8.0	872	368.7	195.4	7.32	0.04	0.04	0.01	10.8	7.6	3.2	511.9	284.4	227.5	522.7	292.0	230.7	4.26	379
02/17/15	4	16.9	0.9	8.0	800	511.0	767.8	8.21	0.00	0.16	0.01	7.6	4.0	3.6	545.7	450.7	95.1	553.3	454.7	98.7	3.96	307
03/17/15	4	12.1	2.9	7.9	828	386.9	237.3	0.76	0.02	0.03	0.00	8.8	7.6	1.2	423.2	272.4	150.8	432.0	280.0	152.0	6.91	1240

04/28/14	5	11.0	17.1	8.6	414	219.0	195.4	3.62	0.05	0.17	0.05										5.71	585
05/19/14	5	10.3	16.8	8.3	448	233.6	181.5	3.97	0.03	0.18	0.07	22.0	11.6	10.4	370.0	220.4	149.6	392.0	232.0	160.0	8.8	1600
06/16/14	5	10.9	22.6	8.0	527	230.0	181.5	8.13	0.04	0.20	0.13	30.0	24.5	5.5	392.7	214.2	178.5	422.7	238.7	184.0	4.87	389
07/14/14	5	8.6	26.1	8.4	507	248.2	209.4	6.37	0.19	0.20	0.11	26.0	22.0	4.0	354.0	216.7	137.3	380.0	238.7	141.3	11.5	2890
08/11/14	5	7.7	24.3	8.4	420	0.0	0.0														3.29	154
09/15/14	5	8.4	18.9	8.4	456	281.1	139.6	0.90	0.33	0.25	0.18	16.8	12.0	4.8	323.2	169.3	153.9	340.0	181.3	158.7	6.42	823
10/13/14	5	11.0	14.3	8.1	605	383.3	418.8	4.23	0.00	0.17	0.08	11.2	9.2	2.0	391.5	182.8	208.7	402.7	192.0	210.7	5.14	492
11/10/14	5	11.1	8.2	8.2	810	357.7	209.4	8.13	0.00	0.11	0.07	10.8	7.2	3.6	413.2	230.1	183.1	424.0	237.3	186.7	3.22	146
12/08/14	5	0.6	2.2	8.0	802	693.5	837.6	4.24	0.00	0.11	0.03	10.4	5.2	5.2	425.6	288.1	137.5	436.0	293.3	142.7	5.2	504
01/20/14	5	0.1	2.9	8.0	872	394.2	195.4	9.33	0.01	0.06	0.11	13.2	7.6	5.6	502.8	291.1	211.7	516.0	298.7	217.3	4.26	379
02/17/15	5	16.7	0.9	8.0	801	620.5	279.2	9.44	0.03	0.05	0.05	6.0	3.2	2.8	419.3	307.5	111.9	425.3	310.7	114.7	3.96	307
03/17/15	5	13.3	2.9	7.9	831	412.5	251.3	0.75	0.02	0.05	0.00	6.8	5.2	1.6	425.2	309.5	115.7	432.0	314.7	117.3	6.91	1240

04/28/14	6	10.9	17.1	8.6	416	197.1	153.6	3.79	0.02	0.17	0.03										5.71	585
05/19/14	6	10.1	16.8	8.4	449	226.3	167.5	4.61	0.02	0.19	0.08	25.2	15.2	10.0	380.1	223.5	156.7	405.3	238.7	166.7	8.8	1600
06/16/14	6	10.6	22.7	8.1	525	233.6	181.5	10.16	0.03	0.19	0.11	29.0	23.0	6.0	400.3	219.7	180.7	429.3	242.7	186.7	4.87	389
07/14/14	6	8.5	26.1	8.4	506	208.1	209.4	7.42	0.19	0.26	0.10	33.0	27.5	5.5	329.7	196.5	133.2	362.7	224.0	138.7	11.5	2890
08/11/14	6	7.3	24.3	8.4	423	204.4	279.2	2.53	0.02	0.13	0.12										3.29	154
09/15/14	6	8.3	18.9	8.5	457	240.9	125.6	1.43	0.25	0.26	0.18	14.8	10.8	4.0	325.2	167.9	157.3	340.0	178.7	161.3	6.42	823
10/13/14	6	10.7	14.3	8.1	604	346.8	349.0	4.77	0.00	0.24	0.08	11.2	8.0	3.2	386.1	193.3	192.8	397.3	201.3	196.0	5.14	492
11/10/14	6	11.0	8.2	8.2	814	376.0	195.4	5.87	0.23	0.11	0.02	8.8	6.4	2.4	412.5	241.6	170.9	421.3	248.0	173.3	3.22	146
12/08/14	6	0.6	2.3	8.3	802	438.0	558.4	3.72	0.00	0.12	0.04	10.0	4.0	6.0	423.3	304.0	119.3	433.3	308.0	125.3	5.2	504
01/20/14	6	0.1	2.9	8.0	874	354.1	167.5	10.15	0.01	0.07	0.13	10.0	6.8	3.2	476.7	293.2	183.5	486.7	300.0	186.7	4.26	379
02/17/15	6	16.5	0.8	8.1	806	474.5	279.2	7.19	0.00	0.10	0.01	8.8	6.0	2.8	464.5	380.7	83.9	473.3	386.7	86.7	3.96	307
03/17/15	6	13.0	2.9	8.0	828	328.5	307.1	0.76	0.02	0.04	0.00	9.2	8.0	1.2	429.5	298.7	130.8	438.7	306.7	132.0	6.91	1240

04/28/14	7	10.9	17.2	8.7	416	215.4	195.4	3.39	0.03	0.18	0.04										5.71	585
05/19/14	7	9.7	16.8	8.5	448	240.9	167.5	4.38	0.01	0.16	0.06	23.2	14.4	8.8	378.1	230.9	147.2	401.3	245.3	156.0	8.8	1600
06/16/14	7	11.1	23.0	8.3	500	222.7	167.5	8.25	0.05	0.16	0.09	24.0	18.0	6.0	369.3	215.3	154.0	393.3	233.3	160.0	4.87	389
07/14/14	7	12.6	26.1	8.4	503	237.3	279.2	9.71	0.23	0.19	0.15	32.0	26.5	5.5	334.7	200.2	134.5	366.7	226.7	140.0	11.5	2890
08/11/14	7	7.2	24.5	8.4	424	211.7	195.4	6.08	0.07	0.06	0.08	6.8	0.4	6.4	277.2	86.3	190.9	284.0	86.7	197.3	3.29	154
09/15/14	7	8.0	19.0	8.4	455	255.5	125.6	1.49	0.34	0.22	0.15	17.6	13.6	4.0	314.4	167.7	146.7	332.0	181.3	150.7	6.42	823
10/13/14	7	11.5	14.3	8.2	601	328.5	349.0	5.17	0.00	0.18	0.07	9.2	6.4	2.8	422.8	212.3	210.5	432.0	218.7	213.3	5.14	492
11/10/14	7	9.5	9.2	8.3	731	295.7	209.4	3.44	0.15	0.12	0.00	10.8	8.0	2.8	367.9	248.0	119.9	378.7	256.0	122.7	3.22	146
12/08/14	7	0.6	2.3	8.3	803	328.5	418.8	4.39	0.06	0.16	0.02	10.0	4.8	5.2	443.3	291.2	152.1	453.3	296.0	157.3	5.2	504
01/20/14	7	0.2	2.8	8.1	875	346.8	279.2	6.84	0.37	0.06	0.10	10.0	8.4	1.6	486.0	295.6	190.4	496.0	304.0	192.0	4.26	379
02/17/15	7	15.5	1.1	8.1	783	511.0	418.8	7.25	0.08	0.04	0.09	8.0	5.6	2.4	434.7	359.7	74.9	442.7	365.3	77.3	3.96	307
03/17/15	7	15.7	2.9	8.1	827	357.7	321.1	0.73	0.00	0.05	0.00	11.2	9.2	2.0	427.5	289.5	138.0	438.7	298.7	140.0	6.91	1240

04/28/14	8	10.9	17.2	8.7	416	178.9	167.5	3.10	0.02	0.20	0.01										5.71	585
05/19/14	8	9.4	16.8	8.5	448	237.3	195.4	3.02	0.03	0.18	0.34	25.2	15.6	9.6	348.1	208.4	139.7	373.3	224.0	149.3	8.8	1600
06/16/14	8	11.0	23.2	8.3	499	233.6	181.5	6.60	0.02	0.14	0.11	22.5	17.5	5.0	346.8	197.2	149.7	369.3	214.7	154.7	4.87	389
07/14/14	8	8.3	26.1	8.4	501	266.5	223.4	6.13	0.19	0.20	0.11	34.5	27.5	7.0	325.5	183.2	142.3	360.0	210.7	149.3	11.5	2890
08/11/14	8	6.5	24.6	8.4	426	237.3	293.2	2.84	0.06	0.15	0.07	3.6	0.4	3.2	281.7	116.9	164.8	285.3	117.3	168.0	3.29	154
09/15/14	8	7.9	18.9	8.4	453	259.2	125.6	1.55	0.26	0.29	0.22	14.8	12.4	2.4	315.9	166.3	149.6	330.7	178.7	152.0	6.42	823
10/13/14	8	10.6	14.3	8.2	601	292.0	349.0	5.41	0.00	0.19	0.13	9.6	8.8	0.8	378.4	191.2	187.2	388.0	200.0	188.0	5.14	492
11/10/14	8	9.3	9.5	8.3	715	313.9	251.3	3.87	0.20	0.29	0.10	10.8	6.0	4.8	366.5	232.7	133.9	377.3	238.7	138.7	3.22	146
12/08/14	8	1.5	2.3	8.3	804	401.5	279.2	3.78	0.03	0.10	0.78	10.0	4.0	6.0	439.3	300.0	139.3	449.3	304.0	145.3	5.2	504
01/20/14	8	1.1	2.8	8.1	872	365.0	111.7	8.99	0.02	0.04	0.00	12.4	9.2	3.2	478.3	278.8	199.5	490.7	288.0	202.7	4.26	379
02/17/15	8	16.1	1.0	8.2	801	547.5	279.2	7.78	0.00	0.10	0.00	6.4	4.8	1.6	437.6	264.5	173.1	444.0	269.3	174.7	3.96	307
03/17/15	8	15.8	3.0	8.0	828	386.9	321.1	0.64	0.00	0.06	0.00	14.8	13.2	1.6	446.5	322.8	123.7	461.3	336.0	125.3	6.91	1240

04/28/14	9	10.3	18.0	8.5	760	226.3	209.4	4.20	0.06	2.11	1.61										5.71	585
05/19/14	9	9.5	16.8	8.4	498	244.6	181.5	4.08	0.04	0.19	0.08	24.0	15.2	8.8	376.0	235.5	140.5	400.0	250.7	149.3	8.8	1600
06/16/14	9	10.9	23.3	8.2	951	226.3	223.4	6.92	0.13	1.54	1.50	20.5	15.5	5.0	623.5	456.5	167.0	644.0	472.0	172.0	4.87	389
07/14/14	9	8.3	26.1	8.4	528	215.4	195.4	5.56	0.21	0.25	0.17	31.5	26.0	5.5	328.5	196.7	131.8	360.0	222.7	137.3	11.5	2890
08/11/14	9	7.9	25.3	8.1	1380	251.9	265.2	6.14	0.27	3.67	3.04	6.8	2.0	4.8	819.9	675.3	144.5	826.7	677.3	149.3	3.29	154
09/15/14	9	7.9	18.9	8.5	453	266.5	125.6	0.77	0.32	0.05	0.00	14.8	9.6	5.2	303.9	178.4	125.5	318.7	188.0	130.7	6.42	823
10/13/14	9	10.3	14.6	8.1	709	310.3	418.8	5.17	0.00	0.70	0.47	9.2	8.8	0.4	436.1	281.9	154.3	445.3	290.7	154.7	5.14	492
11/10/14	9	10.1	10.4	8.2	1068	317.6	181.5	4.20	0.32	1.06	1.10	8.8	5.6	3.2	500.5	387.7	112.8	509.3	393.3	116.0	3.22	146
12/08/14	9	1.9	2.3	8.3	894	365.0	418.8	3.96	0.05	0.25	0.20	7.2	4.4	2.8	447.5	335.6	111.9	454.7	340.0	114.7	5.2	504
01/20/14	9	14.7	3.1	8.1	928	379.6	195.4	7.04	0.01	0.45	0.17	9.2	5.6	3.6	492.1	303.7	188.4	501.3	309.3	192.0	4.26	379
02/17/15	9	15.8	1.6	8.1	894	474.5	418.8	8.55	0.01	0.35	0.28	7.2	5.2	2.0	451.5	364.1	87.3	458.7	369.3	89.3	3.96	307
03/17/15	9	15.8	3.0	8.1	1139	376.0	390.9	0.72	0.01	1.13	1.12	12.4	9.6	2.8	659.6	531.7	127.9	672.0	541.3	130.7	6.91	1240

04/28/14	11	9.1	17.9	8.5	740	226.3	195.4	3.71	0.05	1.60	0.99										5.71	585
05/19/14	11	9.4	17.0	8.5	517	233.6	209.4	3.24	0.02	0.52	0.37	26.4	16.0	10.4	416.3	289.3	126.9	442.7	305.3	137.3	8.8	1600
06/16/14	11	11.1	23.4	8.2	565	259.2	209.4	5.77	0.05	0.76	0.80	24.5	19.0	5.5	522.2	314.3	207.8	546.7	333.3	213.3	4.87	389
07/14/14	11	8.1	26.1	8.4	534	259.2	223.4	5.82	0.19	0.38	0.27	34.0	27.0	7.0	362.0	235.7	126.3	396.0	262.7	133.3	11.5	2890
08/11/14	11	7.7	25.8	8.2	1191	266.5	237.3	4.62	0.15	2.81	2.54				702.8	541.2	161.6	702.8	541.2	161.6	3.29	154
09/15/14	11	7.9	19.1	8.4	549	259.2	153.6	1.30	0.32	0.51	0.77	13.6	8.0	5.6	370.4	252.0	118.4	384.0	260.0	124.0	6.42	823
10/13/14	11	9.1	15.6	8.1	960	529.3	418.8	4.86	0.00	2.60	1.82	10.4	6.4	4.0	580.3	389.6	190.7	590.7	396.0	194.7	5.14	492
11/10/14	11	9.3	11.9	8.2	1391	324.9	195.4	5.14	0.20	2.68	12.48	9.6	5.6	4.0	717.1	589.1	128.0	726.7	594.7	132.0	3.22	146
12/08/14	11	1.1	3.6	8.3	1044	438.0	418.8	4.22	0.00	1.14	0.05	7.6	4.0	3.6	565.7	384.0	181.7	573.3	388.0	185.3	5.2	504
01/20/14	11	13.2	4.7	8.1	1221	350.4	237.3	8.23	0.03	1.33	1.21	6.4	1.2	5.2	653.6	460.1	193.5	660.0	461.3	198.7	4.26	379
02/17/15	11	15.4	2.7	8.1	1200	620.5	418.8	8.30	0.01	1.30	1.28	9.6	6.4	3.2	627.7	556.3	71.5	637.3	562.7	74.7	3.96	307
03/17/15	11	15.0	3.7	8.2	943	335.8	376.9	0.71	0.00	0.21	0.33	9.2	8.4	0.8	562.8	440.9	121.9	572.0	449.3	122.7	6.91	1240
04/28/14	12	11.4	17.9	8.4	671	233.6	223.4	3.73	0.10	1.92	1.19										5.71	585
05/19/14	12	11.8	17.0	8.3	512	248.2	195.4	3.76	0.06	0.51	0.34	197.6	174.4	23.2	370.4	238.9	131.5	568.0	413.3	154.7	8.8	1600
06/16/14	12	11.1	23.3	7.9	691	248.2	195.4	6.79	0.03	1.35	0.74	35.0	28.5	6.5	478.3	287.5	190.8	513.3	316.0	197.3	4.87	389
07/14/14	12	8.4	25.4	8.2	537	237.3	181.5	6.77	0.21	0.42	0.26	60.6	51.3	9.4	347.4	218.1	129.3	408.0	269.3	138.7	11.5	2890
08/11/14	12	7.6	25.6	8.3	973	248.2	321.1	3.88	0.23	2.38	1.68	6.0	2.0	4.0	571.3	416.7	154.7	577.3	418.7	158.7	3.29	154
09/15/14	12	8.0	18.8	8.4	563	262.8	125.6	1.49	0.30	0.88	1.13	17.6	12.4	5.2	381.1	244.9	136.1	398.7	257.3	141.3	6.42	823
10/13/14	12	14.3	15.1	8.3	822	456.3	558.4	5.14	0.00	1.21	0.91	12.0	8.4	3.6	493.3	312.9	180.4	505.3	321.3	184.0	5.14	492
11/10/14	12	9.8	10.6	8.3	1151	324.9	209.4	5.24	0.00	1.33	1.44	11.6	4.4	7.2	565.7	468.9	96.8	577.3	473.3	104.0	3.22	146
12/08/14	12	6.9	3.7	8.4	1014	474.5	558.4	4.52	0.00	0.94	0.13	8.4	2.8	5.6	547.6	363.9	183.7	556.0	366.7	189.3	5.2	504
01/20/14	12	12.8	4.1	8.2	1142	361.4	209.4	7.75	0.03	1.03	1.14	8.4	6.0	2.4	607.6	408.7	198.9	616.0	414.7	201.3	4.26	379
02/17/15	12	15.6	2.4	8.2	1133	474.5	279.2	10.12	0.03	1.12	0.89	8.0	5.2	2.8	592.0	510.8	81.2	600.0	516.0	84.0	3.96	307
03/17/15	12	12.9	3.9	8.2	927	350.4	307.1	0.62	0.11	0.54	0.34	36.4	32.0	4.4	576.9	466.7	110.3	613.3	498.7	114.7	6.91	1240

04/28/14	14	11.8	18.4	8.3	829	251.9	223.4	3.83	0.04	1.96	1.69										5.71	585
05/19/14	14	11.3	17.3	8.3	512	251.9	265.2	5.03	0.02	0.43	0.33	52.0	39.6	12.4	410.7	275.1	135.6	462.7	314.7	148.0	8.8	1600
06/16/14	14	11.2	23.1	7.9	635	251.9	209.4	8.19	0.04	1.12	0.59	47.0	40.0	7.0	719.4	519.7	199.7	766.4	559.7	206.7	4.87	389
07/14/14	14	8.6	26.2	8.3	521	226.3	209.4	5.15	0.18	0.40	0.24	59.5	50.0	9.5	343.2	232.7	110.5	402.7	282.7	120.0	11.5	2890
08/11/14	14	6.6	25.1	8.2	1028	284.7	293.2	4.28	0.12	2.30	1.97	12.8	6.4	6.4	591.2	430.9	160.3	604.0	437.3	166.7	3.29	154
09/15/14	14	7.9	18.6	8.3	543	248.2	139.6	2.55	0.24	0.86	1.07	15.6	11.6	4.0	363.1	237.7	125.3	378.7	249.3	129.3	6.42	823
10/13/14	14	15.4	15.0	8.1	820	383.3	418.8	5.92	0.00	1.54	0.80				495.7	306.9	188.8	495.7	306.9	188.8	5.14	492
11/10/14	14	11.2	9.3	8.4	1042	368.7	237.3	5.44	0.01	1.44	0.84	10.8	4.8	6.0	533.2	421.9	111.3	544.0	426.7	117.3	3.22	146
12/08/14	14	2.8	3.7	8.4	953	401.5	418.8	5.24	0.00	0.91	0.04	8.4	8.4	0.0	539.6	392.9	146.7	548.0	401.3	146.7	5.2	504
01/20/14	14	16.7	4.0	8.2	1158	401.5	223.4	7.66	0.05	1.60	1.45	6.8	5.6	1.2	638.5	463.7	174.8	645.3	469.3	176.0	4.26	379
02/17/15	14	16.4	1.3	8.2	1112	474.5	418.8	9.57	0.03	0.90	0.85	8.4	6.4	2.0	586.3	502.9	83.3	594.7	509.3	85.3	3.96	307
03/17/15	14	13.1	4.0	8.2	961	324.9	349.0	0.62	0.08	0.59	0.34	18.4	16.0	2.4	669.6	570.7	98.9	688.0	586.7	101.3	6.91	1240
Upstream	Mean	9.3	13.2	8.1	620.8	316.3	243.5	4.90	0.08	0.15	0.09	15.0	10.5	4.4	397.5	237.0	160.5	412.3	247.4	164.8		
Down-stream	Mean	10.5	13.9	8.2	860.6	323.9	276.9	4.93	0.09	1.15	1.10	22.5	17.3	5.2	521.4	379.4	142.0	542.9	395.9	147.0		

Table 2. Summary of Illinois RiverWatch-level identifications of macroinvertebrates sampled in seven sites in the Sangamon River in summer 2014. Tolerance values can range from 0 (intolerant) to 11 (tolerant).

		Site							
		Upstream				**Downstream**			
Taxa	**Tolerance Value**	**3**	**5**	**7**	**8**	**11**	**12**	**14**	**Total**
Aquatic worm	10.0	13	1	26	13	10	4	2	**69**
Leech	8.0	2	2	-	1	-	-	1	**6**
Sowbug	6.0	-	1	-	-	-	-	-	**1**
Scud	4.0	1	41	13	1	-	-	1	**57**
Dragonfly	4.5	-	1	-	1	-	-	-	**2**
Broadwinged Damselfly	3.5	-	-	-	-	-	-	2	**2**
Narrowwinged Damselfly	5.5	-	6	8	6	2	-	2	**24**
Torpedo Mayfly	3.0	-	1	-	1	1	-	3	**6**
Swimming Mayfly	4.0	-	-	-	-	-	12	-	**12**
Clinging Mayfly	3.5	1	1	4	8	4	7	33	**58**
Crawling Mayfly	5.5	1	13	26	3	-	-	6	**49**
Stonefly	1.5	-	-	-	7	1	3	7	**18**
Hydropsychid Caddisfly	5.5	-	4	20	89	142	195	203	**653**
Saddle Case Caddisfly	3.0	-	-	-	-	-	-	1	**1**
Other Caddisfly	3.5	-	1	-	-	-	-	-	**1**
Riffle Beetle	5.0	-	-	-	-	-	-	1	**1**
Whirligig Beetle	4.0	-	-	-	6	-	-	1	**7**
Biting Midge	5.0	1	-	1	-	-	-	-	**2**
Bloodworm Midge	11.0	24	-	2	2	1	-	-	**29**
Midge	6.0	245	235	218	218	152	57	38	**1163**
Blackfly	6.0	-	1	1	5	-	2	1	**10**
Left-handed Snail	9.0	11	6	7	1	2	-	-	**27**
Right-handed Snail	7.0	2	-	-	-	-	-	-	**2**
Planorbid Snail	6.5	2	-	-	-	-	-	-	**2**
Limpet	7.0	-	-	-	1	-	-	1	**2**
Total		**303**	**314**	**326**	**363**	**315**	**280**	**303**	**2204**

Table 3. Summary of fish species sampled using seine pulls upstream and downstream of the Sanitary District of Decatur in the Sangamon River on 22 September 2014.

	Site							
Species	**3**	**5**	**7**	**8**	**11**	**12**	**14**	**Total**
Cyprinella lutrensis (Red Shiner)	1	-	-	-	-	-	-	**1**
Cyprinella spiloptera (Spotfin Shiner)	-	1	19	3	6	25	-	**54**
Cyprinella whipplei (Steelcolor Shiner)	-	-	37	-	10	31	14	**91**
Cyprinidae (Minnows)	-	-	2	-	-	9	5	**16**
Dorosoma cepedianum (Gizzard Shad)	-	1	1	-	6	-	-	**8**
Etheostoma nigrum (Johnny Darter)	-	-	-	-	4	-	-	**4**
Fundulus notatus (Blackstripe Topminnow)	1	-	-	-	-	-	-	**1**
Gambusia affinis (Mosquitofish)	-	-	1	1	-	2	3	**7**
Labidesthes sicculus (Brook Silverside)	-	7	-	-	3	-	-	**10**
Lepomis sp. (Sunfishes)	-	-	-	1	11	1	-	**13**
Lepomis cyanellus (Green Sunfish)	-	-	-	2	-	-	1	**3**
Lepomis macrochirus (Bluegill)	11	5	1	2	51	-	2	**72**
Micropterus salmoides (Largemouth Bass)	1	-	-	-	-	-	-	**1**
Notropis atherinoides (Emerald Shiner)	-	-	1	-	-	-	-	**1**
Notropis blennius (River Shiner)	-	-	6	-	-	2	2	**10**
Notropis ludibendus (Sand Shiner)	-	-	5	-	1	24	2	**32**
Pimephales notatus (Bluntnose Minnow)	-	-	-	1	8	1	1	**11**
Pimephales vigilax (Bullhead Minnow)	1	1	-	-	2	-	-	**4**
Pomoxis annularis (White Crappie)	-	2	-	-	-	-	-	**2**
Total	**14**	**17**	**73**	**10**	**102**	**96**	**29**	**341**

Table 4. Catch per unit effort (CPUE) for fish species sampled using seine pulls upstream and downstream of the Sanitary District of Decatur in the Sangamon River on 22 September 2014.

Reach	**Site**	**CPUE (fish per seine pull)**
Upstream	3	7.0
	5	8.5
	7	36.5
	8	5.0
		Upstream Mean = 14.3
Downstream	11	51.0
	12	48.0
	14	14.5
		Downstream Mean = 37.8

Table 5. Asian Carp catches by month and site, 2014. Silver Carp represented, Bighead Carp count in parentheses.

	April	May	June	July	August	September	October
Total	17	31	48	12	64	41	7
SG1	16	13	25(2)	3	26	18	2
SG3	1	18	21	8(1)	38	23	5

Table 6. Larval fish counts by family sampled in 2014 in the Sangamon River at the downstream site SG3.

Family	Total
Catostomidae	33
Centrarchidae	1
Clupeidae	54
Cyprinidae	44
Ictaluridae	1
Lepisosteidae	1
Moronidae	52
Percidae	3
Total	189

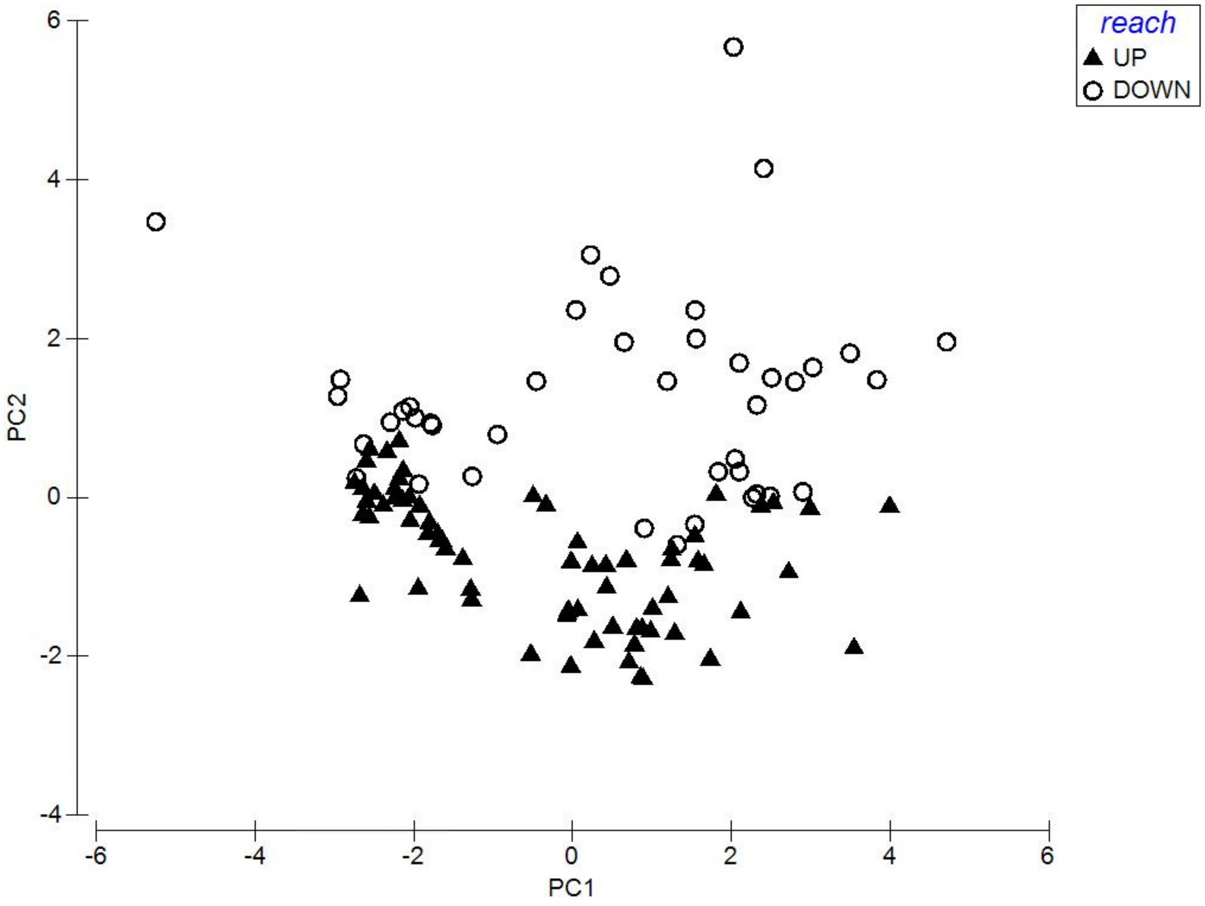

Figure 1. Principle components analysis of water quality data sampled during 2014-2015 from all mainstem sites of the Sangamon River. PCA extracted five factors which account for a total of 76.8 % of the variation in the data. Variation in factor 1 is largely due to conductivity (r = 0.420), hardness (r = 0.395), temperature (r = -0.392), and fixed dissolved solids (r = 0.360), whereas factor 2 is heavily influenced by total phosphorus (r = 0.562). Samples collected from the downstream and upstream were significantly different (ANOSIM, Global R = 0.246, p < 0.001) with more profound differences observed during periods of low reservoir discharge.

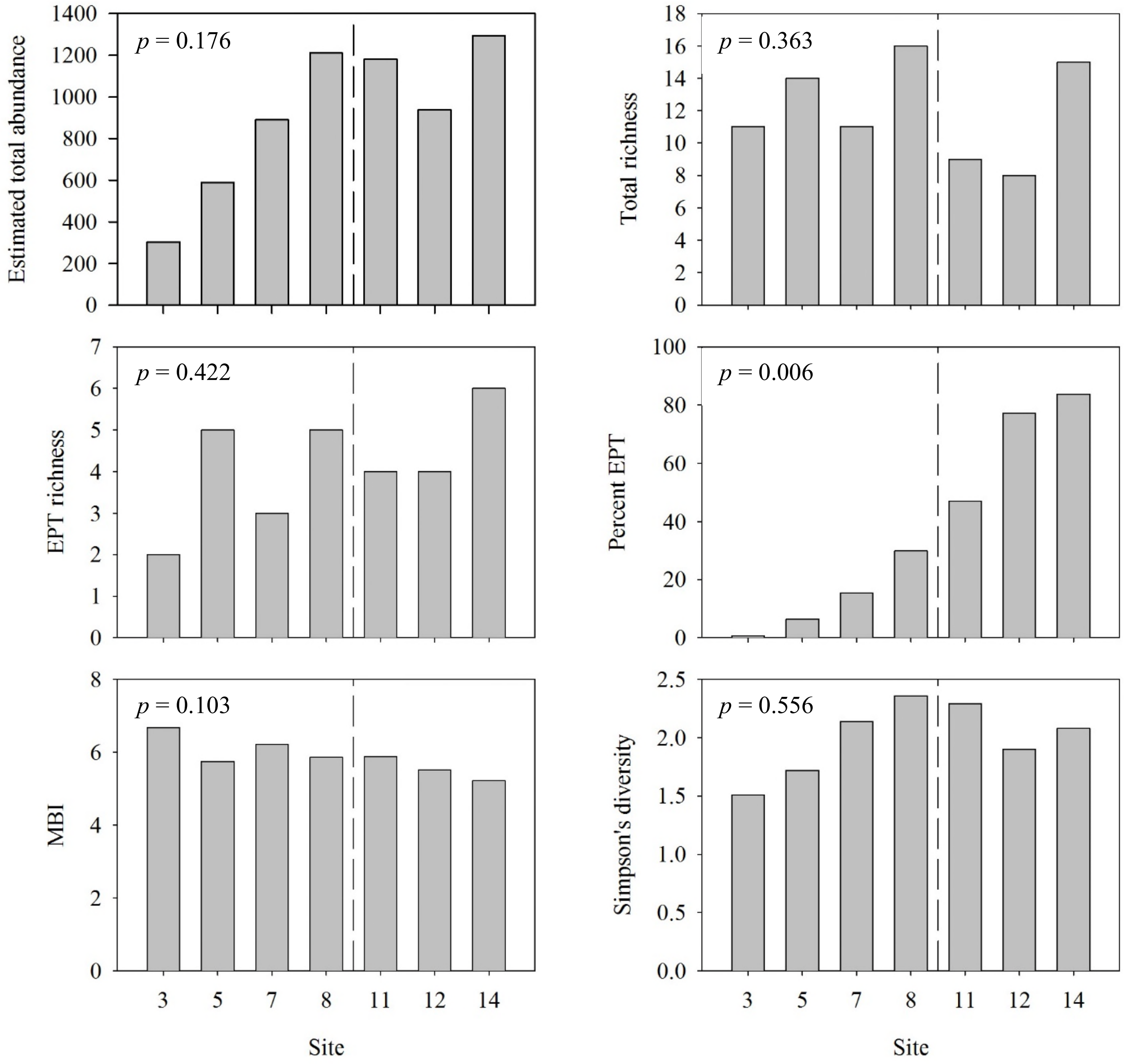

Figure 2. Comparison of macroinvertebrate metrics in seven sites of the Sangamon River in summer 2014. Sites 3, 5, 7, and 8 are upstream of the main effluent outfall, and sites 11, 12, and 14 are downstream of the main effluent outfall. *P*-values compare the upstream reach to downstream reach. The percent EPT was significantly higher in the downstream reach ($F_{1,5}$ = 21.69; p = 0.006).

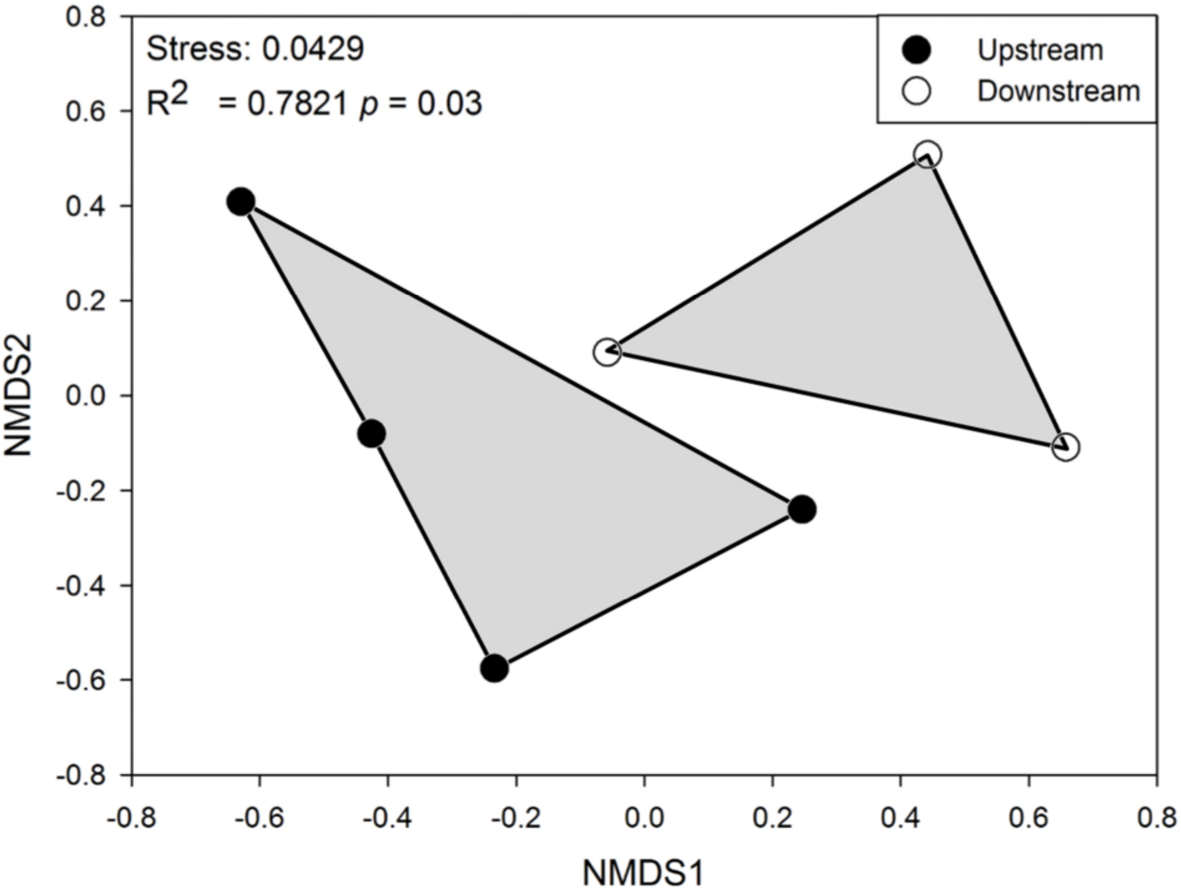

Figure 3. Non-metric multidimensional scaling (NMDS) plot of macroinvertebrate communities based on Bray-Curtis dissimilarities for square root transformed, double standardized count data for seven sites on the Sangamon River sampled during summer 2014. Stress = 0.043. Differences in assemblage structure is shown between reaches ($p = 0.028$).

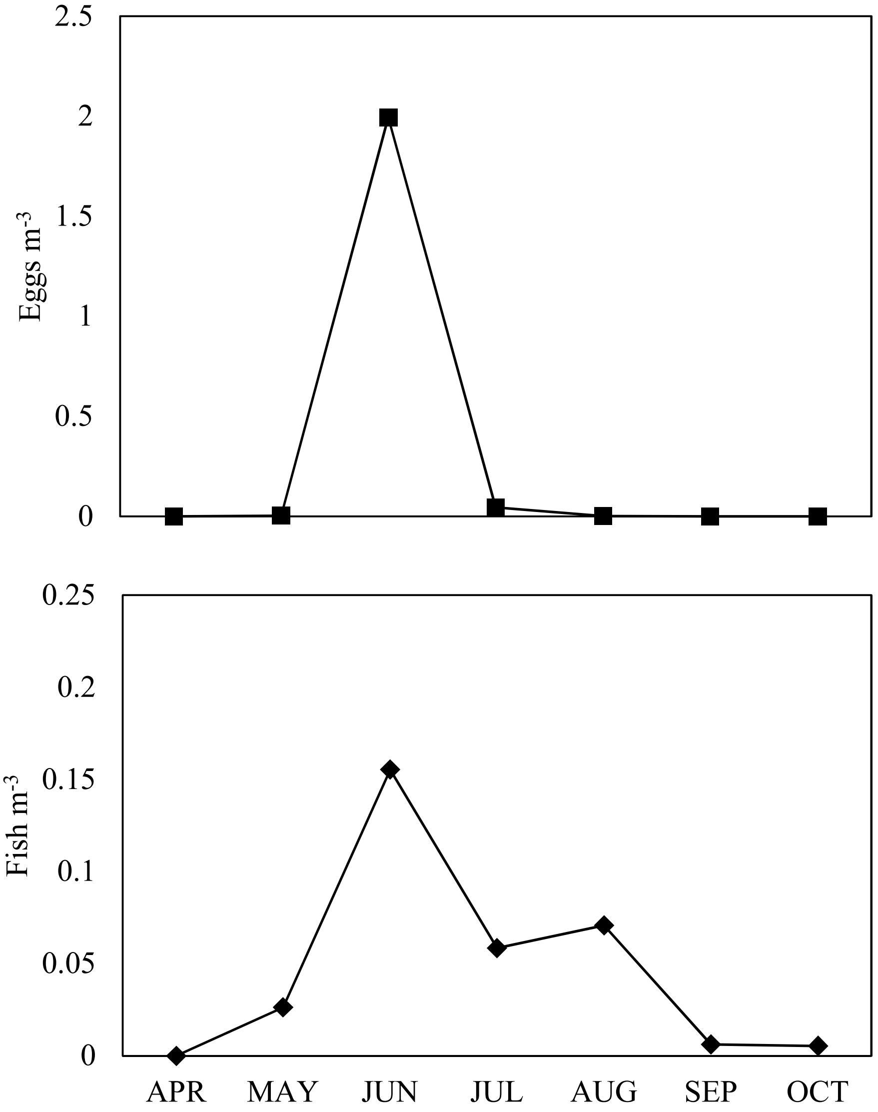

Figure 4. Egg and larval fish densities by month at the downstream site SG3 in the Sangamon River in 2014. Egg densities are depicted in the top graph, larval fish densities are in the bottom graph. Densities are calculated as number/cubic meter of water sampled.

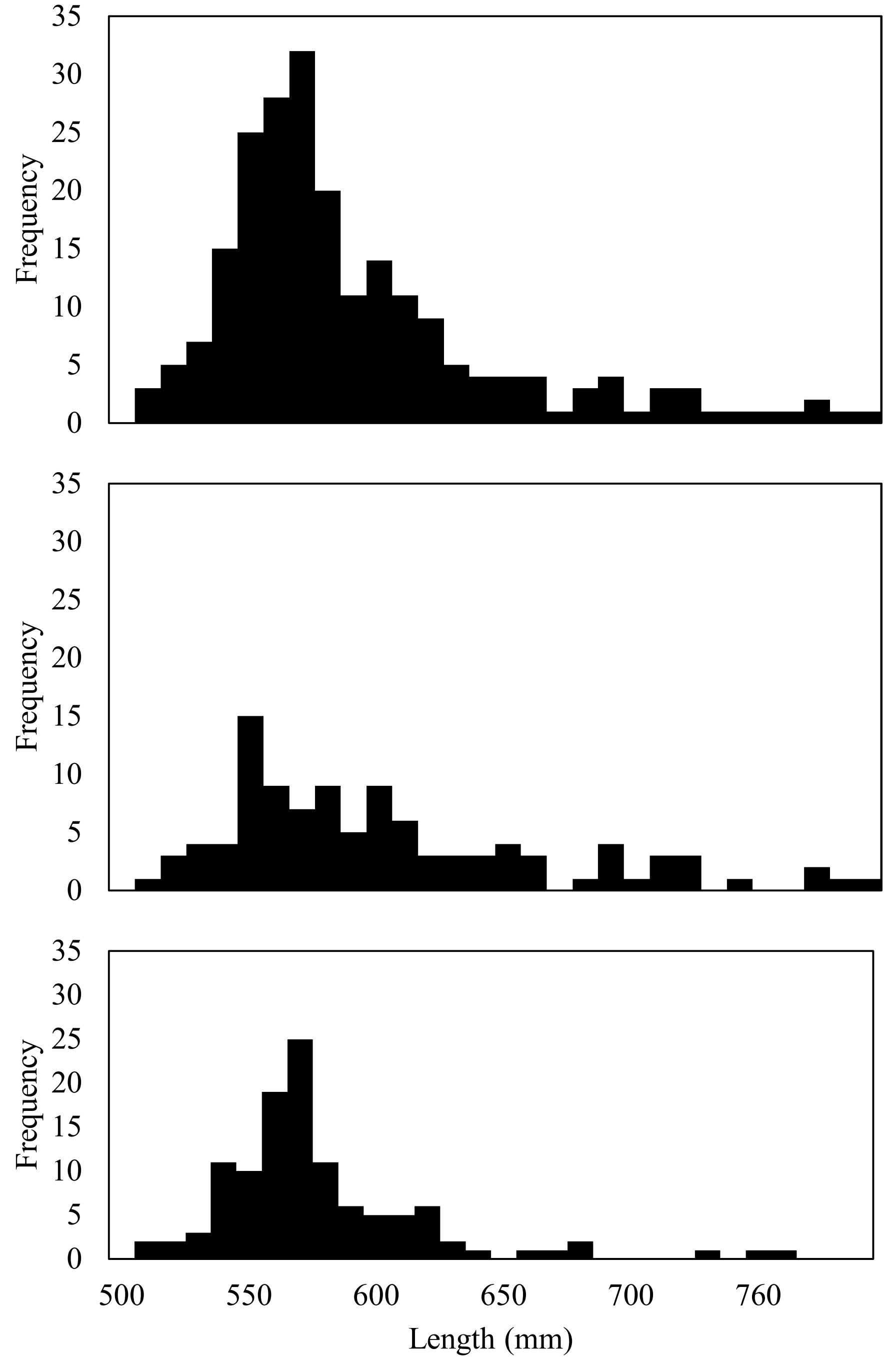

Figure 5. Length frequency of Asian Carp in the Sangamon River, 2014. Total frequency on top, upstream site SG1 frequency in middle, and downstream site SG3 frequency on bottom.

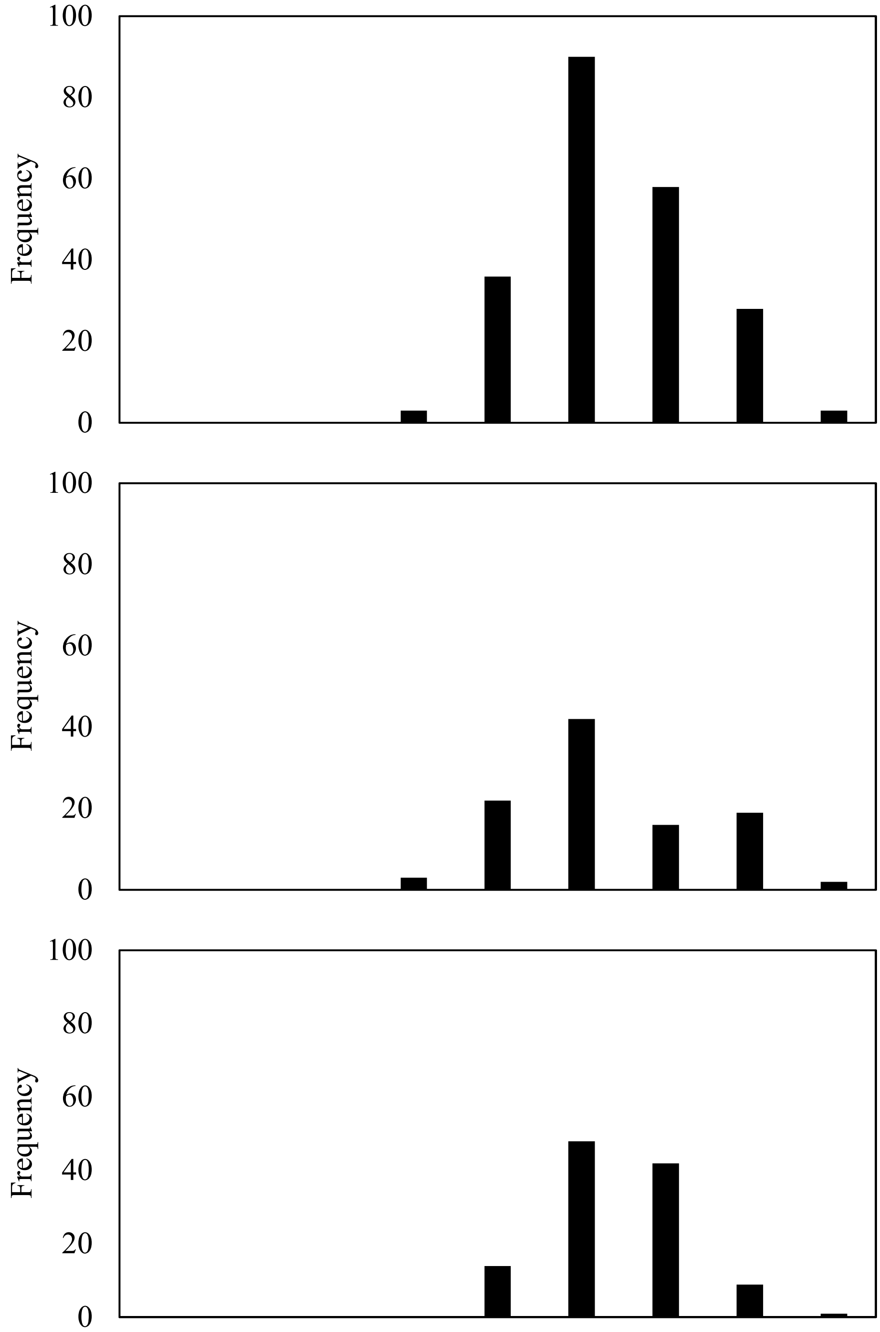

Figure 6. Age frequency of Asian Carp in the Sangamon River, 2014. Total frequency on top, upstream site SG1 frequency in middle, and downstream site SG3 frequency on bottom.

APPENDIX

Sangamon River sites (Site # based on previous studies)

Site 1 – Lincoln Park CSO – above outfall
Site 3 – Lincoln Park CSO – below outfall
Site 4 – Oakland CSO (Lincoln Park) - above outfall
Site 5 – Oakland CSO (Lincoln Park) – below outfall
Site 6 – 7th Ward CSO (End Sunset Dr.) – above outfall
Site 7 – 7th Ward CSO (End Sunset Dr.) - below outfall
Site 8 – Main Treatment Plant (Off Main street) – upstream of main outfall
Site 9 – Main Treatment Plant (Off Main street) –down stream of main outfall
Site 11 – Sangamon River directly downstream of Stevens Creek
Site 12 – Bridge on Wyckles Road
Site 14 – Lincoln Trail Homestead State Park

Routine collections for water quality assessment were conducted at all sites.

Macroinvertebrates and fish were collected form Sites 3, 5, 7, 8, 11, 12, and 14.

Asian Carp were sampled at one site below Lake Decatur Dam and one site downstream near Chandlerville.