

Exhibit 28

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

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for the Sanitary District of Decatur*



TABLE OF CONTENTS

List of Figures	2
List of Tables	2
Introduction	3
Water Effect Ratio Calculation	3
Supporting Information for the Ni WER using the Ni BLM	9
Conclusions	12
Appendix 1 - OSU Ni Toxicity Test	13
Appendix 2 - ANCOVA Analysis	247
Literature Cited	251

LIST OF FIGURES

Figure 1. Histogram of DOC slopes.....	7
Figure 2. Nickel speciation predicted by the BLM over a range of DOC concentrations.....	11
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).....	250

LIST OF TABLES

Table 1 Reported DOC values reported in Sangamon River samples from downstream of the SDD main plant. DOC values that are reported at a detection limit are identified with a "<" in the "DOC as reported" column. Values reported at the detection limit are divided by 2 prior to their use in the WER calculation, as shown in the "DOC used in WER" column.	8
Table A2-2. DOC and Ni effect concentrations used in the ANCOVA analysis.	247
Table A2-3. Results of the ANCOVA analysis.....	248
Table A2-4. Summary of statistical results from the ANCOVA analysis.	248

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. There are relatively few data, however, that show the changing response of an organism to Ni toxicity over a range of DOC values. In a review of the scientific literature on Ni toxicity, Santore et al (2017) documented several published studies that looked at the effect of DOC on Ni toxicity. These included the study by Hoang and the study by Kozlova (Hoang, Tomasso et al. 2004, Kozlova, Wood et al. 2009). An additional study by Doig and Liber (2006) was for an insensitive organism to Ni toxicity and was not considered. The sensitivity of the organism is an important consideration, since the BLM analysis for Ni indicates that the response of Ni toxicity to changes in DOC can vary with Ni concentrations with lower responses seen at higher Ni concentrations. Since insensitive organisms, by definition, require higher Ni concentrations to demonstrate a toxic effect, then insensitive organisms may also show a lower response to DOC. A good example of this effect is seen in the data from Hoang and others (2004). Hoang measured the influence of DOC on nickel toxicity to two different ages of fathead minnow. Young fish were much more sensitive to Ni toxicity and for these fish a response to DOC was observed. Older fish were much less sensitive to Ni toxicity and in these fish no DOC response was observed. This comparison is a good example of how, all other factors being equal (i.e., same test species in the same conditions, the only difference here is the age of the organism and the resulting change in sensitivity to Ni), the sensitivity of the organism can be an important factor in determining whether a response to DOC is observed, as well as the magnitude of the response. The BLM includes a comprehensive description of the chemistry of DOC which automatically adjusts DOC binding in response to changing Ni concentrations. In the BLM, the interactions between DOC and Ni are modeled with over 120 individual reactions that represent a wide range of potential binding sites. The simple DOC equation used in this analysis, however, does not have a

provision for a similar level of complexity. It is important, therefore, to base the DOC slope on data that are relevant for setting the Ni standard.

For the purpose of determining the slope of the relationship between Ni toxicity and DOC, the most relevant data are the chronic *C. dubia* toxicity data reported by OSU. These data are the most relevant, because *C. dubia* is among the most sensitive organism in the freshwater Ni toxicity data (Santore et al, 2017), and as a result Ni toxicity to *C. dubia* occurs at values that are similar to the chronic Ni standard. Since there are few data for *C. dubia* in the OSU study, it is important to demonstrate that the observed effect of DOC on Ni toxicity is supported by other datasets. To that end, additional toxicity data were compared with the DOC response in the OSU study to determine if an overall DOC relationship that is consistent with effects observed in multiple studies and with multiple organisms is possible.

Additional toxicity data from the Santore et al (2017) review were considered for inclusion in the DOC analysis. Ideally, we would have data for other sensitive organisms since, as noted above, insensitive organisms are likely to exhibit less of a response to DOC. From the literature available in Santore et al (2017) the experiments that corresponded to the most sensitive organisms available were considered. The most comparable literature data are the *D. pulex* study by Kozlova et al (2009) and the Hoang et al (2004) tests with the young (<24 hour old) fathead minnow. The data from Hoang et al (2004) were further restricted to the high pH exposures, since these exposures were more like the high pH conditions in the Sangamon River, and pH can affect DOC response (Santore et al, 2017). The OSU, Kozlova et al, and Hoang et al data were used in an ANCOVA analysis to develop an overall DOC equation for the Sangamon River (Appendix 2). The intercept for the OSU dataset with the ANCOVA slope is listed in Appendix 2 as -2.07852 (Table A2-2). Converting from mg/L to µg/L involves simply adding 3, which to four significant digits results in an intercept of 0.9215. The resulting equation is:

$$\log_{10} Ni EC20 = 0.3260 * \log_{10} DOC + 0.9215$$

To evaluate this equation, especially in regards to conformance with the response seen in the OSU data, the ANCOVA slope was compared with the response seen in the OSU toxicity data. The reported Ni EC20s in the OSU study, include confidence intervals around each data point. A Monte Carlo analysis was used to vary Ni EC20 values in order to estimate the uncertainty in the DOC slope represented in the OSU data. Ni EC20 values were randomly generated from a log-normal distribution at each point, with the reported value taken as the mean and the standard deviation derived such that

the distribution would conform to the reported confidence intervals. A histogram of DOC slopes that result from this analysis is shown in Figure 1. The median slope from the Monte Carlo analysis agrees very well with the slope calculated directly from the two EC20 values (dashed orange line, and orange asterisk in Figure 1). The ANCOVA slope is within the confidence bounds on the slope calculated by Monte Carlo (Figure 1). From this comparison, we conclude that the ANCOVA slope is consistent with the DOC response seen in the OSU data. This comparison, along with the ANCOVA results that indicate that the individual slopes in these datasets are not different, supports the use of the ANCOVA slope in the overall DOC equation. As a further comparison, the shaded region in Figure A2-1 shows how uncertainty in the slope relates to the predicted DOC response. The ANCOVA regression equation using the pooled slope from the OSU, Kozlova, and Hoang data lies within the shaded region, which again indicates that the DOC equation determined by ANCOVA with data for three sensitive aquatic species is consistent with the observed trends in the OSU study, and the ANCOVA slope is within the confidence interval of the response in the OSU study.

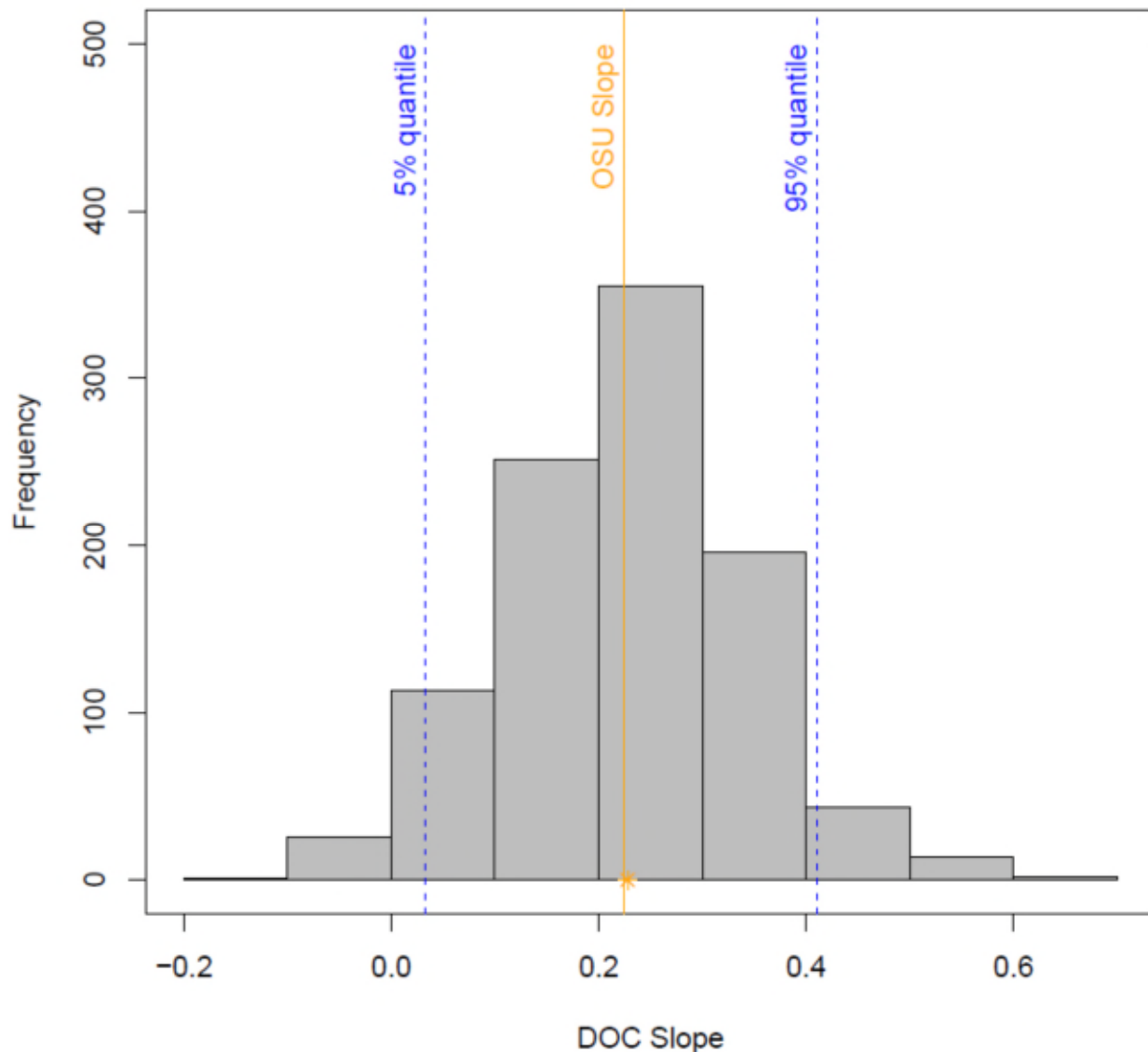


Figure 1. Variation in the slope of the DOC response indicated in the OSU data when uncertainty in the reported Ni EC20 for reproduction is considered by a Monte Carlo analysis. The vertical orange line is the median response from the Monte Carlo analysis. The orange asterisk on the x-axis is the slope derived from the two OSU points without consideration of uncertainty. The dashed vertical blue lines represent the 5th and 95th quantiles, and the region between these quantiles represents a confidence interval around the median slope.

The DOC equation that results from the ANCOVA slope 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. This value is representative of the types of test waters used to derive the Illinois Ni guideline. For

example, the three most sensitive species in the Ni guideline are *C. dubia*, *Brachydanio*, and *D. magna*. The *C. dubia* tests were conducted in dilute mineral water which would have a similarly low DOC concentration. The *Brachydanio* tests were conducted in ultrapure laboratory water, which likewise would be expected to have low or no DOC (the fish *Brachydanio* is now called *Danio rerio*). Finally, the *D. magna* tests were conducted in water from Lago Maggiore. Although DOC was not measured in the test waters used for the Ni study, extensive measurements of organic carbon in Lago Maggiore were reported by Bertoni et al (2004). A median total organic carbon for Lago Maggiore was reported as 0.9 mg/L and that was characterized as “mostly particulate” (Bertoni et al, 2004). Therefore, Lago Maggiore would also be expected to have very low DOC, comparable to the 0.5 mg/L reported in the OSU tests.

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; 2017) and are reproduced again here for supporting the WER calculation (Table 1).

Table 1 Reported DOC values reported in Sangamon River samples from downstream of the SDD main plant. DOC values that are reported at a detection limit are identified with a “<” in the “DOC as reported” column. Values reported at the detection limit are divided by 2 prior to their use in the WER calculation, as shown in the “DOC used in WER” column.

Sampling Site	Sampling Date	DOC as reported (mg/L)	DOC used in WER (mg/L)
1/14/2010	Lincoln Homestead	<5.10	2.55
2/11/2010	Lincoln Homestead	<5.10	2.55
3/4/2010	Lincoln Homestead	<5.10	2.55

4/15/2010	Lincoln Homestead	<5.10	2.55
5/13/2010	Lincoln Homestead	<5.10	2.55
6/10/2010	Lincoln Homestead	<5.10	2.55
7/8/2010	Lincoln Homestead	<5.10	2.55
8/12/2010	Lincoln Homestead	<5.10	2.55
8/26/2010	Lincoln Homestead	10.00	10.00
9/9/2010	Lincoln Homestead	6.95	6.95
7/28/2011	Lincoln Homestead	6.00	6.00
8/4/2011	Lincoln Homestead	7.00	7.00
8/9/2012	Lincoln Homestead	13.00	13.00
8/23/2012	Lincoln Homestead	17.00	17.00
8/26/2010	Rock Springs	12.00	12.00
9/9/2010	Rock Springs	10.00	10.00
8/9/2012	Rock Springs	18.00	18.00
8/23/2012	Rock Springs	23.00	23.00
8/26/2010	RD at Rock Springs B	12.00	12.00
8/26/2010	RD at Lincoln Homestead	10.00	10.00
9/9/2010	RD at Rock Springs B	10.00	10.00
9/9/2010	RD at Lincoln Homestead	7.90	7.90
	Average of Downstream Samples		8.33
	Reference		0.5

The DOC data from monitoring in the Sangamon downstream of the main plant result in an average DOC concentration of 8.33 mg/L. Using this average DOC value with the relationship between DOC and Ni EC20 results in an average Ni EC20 of 16.662 µg/L. The same relationship applied to a reference sample with a DOC of 0.5 mg/L would have a corresponding Ni EC20 of 6.663 µg/L. Substituting these Ni EC20 values in the WER equation yields a WER value of $16.662/6.663 = 2.50$.

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., 2017). The Ni BLM is useful here as an independent line of evidence for assessing how chemical factors in the Sangamon River

affect Ni toxicity. The BLM is based on mechanistic relationships. For example, the BLM simulates the effects of NOM on Ni toxicity by considering to what extent NOM is expected to chemically interact with Ni, based on thermodynamic information (Santore et al., 2017). The BLM, therefore, predicts NOM effects based on the theoretical interactions between NOM and Ni.

The calibration and validation of the Ni BLM has included comparison with hundreds of individual toxicity tests in acute and chronic exposures from laboratories around the world (Santore et al, 2017). The BLM analysis indicates that diverse aquatic life including fish and invertebrates exhibit consistent responses in Ni toxicity to the variation in water chemistry. The most important toxicity modifying factors identified in the BLM analysis are natural organic matter (quantified as DOC) and hardness. From the standpoint of developing a site-specific guideline for Ni, the effect of hardness is already included in the Illinois Ni water quality guideline. Therefore, a site-specific adjustment needs to only consider the additional effects of DOC on Ni toxicity. The BLM analysis, therefore, supports the conclusion in this report that a WER multiplier that includes DOC effects, coupled with the Illinois criterion equation which considers hardness effects, will consider the two most important factors in understanding Ni bioavailability.

The DOC effect that is predicted in the BLM results from the chemical interactions between DOC and dissolved Ni. Natural organic matter is a complex assemblage of molecules that include reactive functional groups capable of binding Ni. The BLM considers interactions between organic matter and Ni and predicts how the presence of organic matter will affect the chemical speciation of Ni. For example, in Figure 2, the effect of DOC on Ni speciation is illustrated. At low DOC, most of the dissolved Ni is in the form of inorganic complexes which includes the bicarbonate and carbonate complexes, Ni hydroxide, and the free Ni ion. As DOC increases, the amount of Ni bound to DOC also increases. At DOC concentrations representative of the Sangamon River, as much as 70% of the dissolved Ni is expected to be bound to DOC, with corresponding reductions in inorganic Ni, and free Ni ion (Figure 2). Since the free Ni ion represents the most bioavailable form of Ni, the reduction from around 78% to 25% free Ni suggests that bioavailability has been reduced by about a factor of 3.

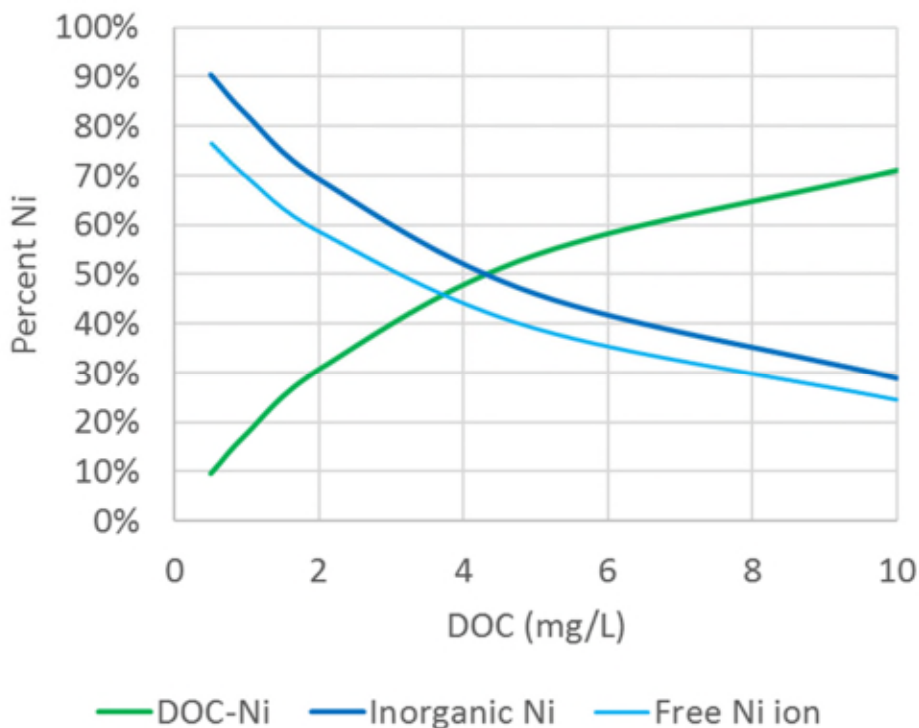


Figure 2. Nickel speciation predicted by the BLM over a range of DOC concentrations.

The Ni BLM has been used to estimate a WER for the Sangamon River (Santore 2018). A special sampling effort was conducted in 2010 to provide measurements for all of the required BLM parameters (i.e., temperature, pH, DOC, calcium, magnesium, sodium, potassium, sulfate, chloride, and alkalinity). If the Ni BLM is applied to these samples the estimated WER is 2.58. The average DOC for these special study samples happens to be different than the average DOC in Table 1. Since DOC is the primary factor affecting Ni bioavailability, the different DOC values in these two analyses may confound their comparison. To provide a clearer comparison between the BLM and the DOC-equation results, the BLM can be applied to average chemistry, including the average DOC in Table 1. When applied to average chemistry, the estimated WER is 2.63. Both of these WER values are in excellent agreement with the WER derived from the OSU toxicity tests. These values are also consistent with the approximately 3-fold reduction in bioavailability that would be expected given the effects of DOC on Ni speciation shown in Figure 2. This level of agreement indicates that the NOM effects that are directly observed in the OSU study are consistent with the theoretical effects that would be expected, based on the thermodynamic interactions simulated in the BLM. The good correspondence between these two methods indicates that the derived WER for Ni in the Sangamon is reasonable, and is supported by a mechanistic understanding of the interactions between NOM and Ni.

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 2.50. 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 2.63. The excellent agreement between these results provides supporting evidence that the WER of 2.50 is a reasonable and defensible result for the Sangamon River.

APPENDIX 1 – OSU NI TOXICITY TEST REPORTS



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

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2017. Chronic toxicity of a nickel-spiked simulated effluent,
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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

ABSTRACT	iii
SIGNATURE PAGE	vi
INDIVIDUAL TEST PERSONNEL PAGE	vii
QUALITY ASSURANCE STATEMENT	viii
1. INTRODUCTION	1-1
2. MATERIALS AND METHODS	2-1
2.1 METHODOLOGY	2-1
2.2 TEST SUBSTANCE.....	2-1
2.3 DISSOLVED ORGANIC CARBON	2-1
2.4 TEST SYSTEM	2-1
2.4.1 Species	2-1
2.4.2 Source	2-1
2.4.3 Culture Acclimation	2-1
2.5 DILUTION WATER.....	2-2
2.5.1 Simulated Effluent/Laboratory Water Preparation	2-2
2.6 ROUTE OF EXPOSURE AND SELECTION OF TEST CONCENTRATIONS.....	2-3
2.7 TEST CHAMBERS.....	2-3
2.8 TEST CONDITIONS	2-3
2.9 TEST INITIATION, RENEWAL, AND FEEDING.....	2-3
2.10 TEST MONITORING	2-4
2.10.1 Water Quality	2-4
2.10.2 Biological Monitoring.....	2-4
2.10.3 Analytical Sampling.....	2-4
2.11 ANALYTICAL CONFIRMATION.....	2-5
2.12 STATISTICAL ANALYSIS	2-5
3. RESULTS AND CONCLUSIONS	3-1
3.1 TEST CONDITIONS	3-1
3.2 DEFINITIVE TEST CONCENTRATIONS.....	3-5
3.3 BIOLOGICAL RESULTS.....	3-10
3.4 CONCLUSIONS.....	3-14
3.5 PROTOCOL DEVIATIONS AND AMENDMENTS	3-15
3.6 LOCATION OF RAW DATA ARCHIVES	3-15
4. REFERENCES	4-1

LIST OF TABLES

2-2 Target simulated effluent water quality before and after dilution 2-2
3-1 Summary of Water Quality Parameters - Toxicity testing 3-3
3-2 Summary of Water Quality Parameters – Control/dilution water 3-4
3-3 Water quality – Dissolved Organic Carbon 3-4
3-4 Summary of Metal Analyses – Simulated Effluent w/o DOC - Total 3-6
3-5 Summary of Metal Analyses – Simulated Effluent w/o DOC - Dissolved ... 3-7
3-6 Summary of Metal Analyses – Simulated Effluent with DOC - Total 3-8
3-7 Summary of Metal Analyses – Simulated Effluent with DOC - Dissolved... 3-9
3-8 Summary of Biological – Simulated Effluent without DOC 3-11
3-9 Summary of Biological – Simulated Effluent with DOC 3-12
3-10 Summary of Statistics 3-13
3-11 Nickel Biotic Ligand Model (BLM) Predicted versus Observed 3-14

APPENDICES

- A Protocol
- B Metal Data Summaries
- C Water Quality Summaries
- D Raw Data

SIGNATURE PAGE

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USA

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

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Principal Investigator Signature: _____
William Stubblefield, Ph.D. Date

Study Director Signature: _____
Allison Cardwell Date

INDIVIDUAL TEST PERSONNEL PAGE

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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 ($\text{NiCl}_2 \times 6\text{H}_2\text{O}$; 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 $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ 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 ($\mu\text{g/L Ni}$).

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 (CaSO₄ • 2H₂O, MgSO₄, KCl, and NaHCO₃) 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 B₁₂ 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 (CaSO₄ • 2H₂O) to deionized water and mixed overnight.
- Addition of reagent grade salt (MgSO₄) to deionized water and mixed overnight.
- Addition of reagent grade salts (KCl, NaCl, and NaHCO₃) to deionized water and mixed overnight.
- Solutions combined and mixed.
- Solution bubbled with CO₂ to reduce pH and promote carbonate dissolution. pH reduced to below 6.0. Water left in a zero headspace container overnight.
- Solution bubbled with O₂ 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 CaCO₃) (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 $\text{pH} < 2$ and refrigerated ($0 - 4\text{ }^{\circ}\text{C}$) 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 ($\text{LC}_{10}/\text{LC}_{20}/\text{LC}_{50}$ and $\text{EC}_{10}/\text{EC}_{20}/\text{EC}_{50}$). 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 ($25 \pm 1^\circ\text{C}$) 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 <u>without</u> 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 <u>with</u> 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 <u>without</u> DOC (Ni WER 1132R CDC)	Simulated Effluent <u>with</u> DOC (Ni WER 1126 CDC)	Previous Study Simulated Effluent <u>without</u> 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)						Average ± SD
	0 (Control)	12.2 µg/L Ni	17.4 µg/L Ni	0 (Control)	12.2 µg/L Ni	17.4 µg/L Ni	
	new			old			
DOC (mg/L) :	< 0.20 ¹	Not sampled	< 0.20 ¹	0.93	Not sampled	1.02	0.54 ± 0.51 *

Parameter	Simulated Effluent with DOC (Ni WER 1126 CDC)						Average ± SD
	0 (Control)	26.9 µg/L Ni	38.5 µg/L Ni	0 (Control)	26.9 µg/L Ni	38.5 µg/L Ni	
	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 - ($\mu\text{g/L Ni}$)

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

¹Very-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

¹Very-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 - ($\mu\text{g/L Ni}$)

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 ($\geq 80\%$ survival and $\geq 60\%$ 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 $\mu\text{g/L}$ dissolved Ni and above in the simulated effluent “without DOC” test and a LOEC of 32.5 $\mu\text{g/L}$ dissolved Ni in the “with DOC” test) were not used in the NOEC/LOEC determinations for reproduction, resulting in reproductive LOEC of > 8.0 $\mu\text{g/L}$ dissolved Ni in the “without DOC” test and a LOEC of 17.4 $\mu\text{g/L}$ 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 LC₂₀ of 8.7 – 11.5 and an EC₂₀ of 4.8 – 6.4 µg/L Ni for survival and reproduction, respectively. This is compared with the observed effect of a survival LC₂₀ of 8.3 µg/L dissolved Ni and a reproductive EC₂₀ 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 EC₂₀ of 7.4 µg/L dissolved Ni which was very similar to the reproductive EC₂₀ achieved in this study (i.e., EC₂₀ 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 LC₂₀ of 60.9 – 71.2 and an EC₂₀ of 34.8 – 40.8 µg/L dissolved Ni compared to the observed LC₂₀ of 26.3 and EC₂₀ 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 performed 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 3rd 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

- American Public Health Association (APHA). 2012. Standard Methods for the Examination of Water and Wastewater, 22nd edition. Washington, D.C.
- Comprehensive Environmental Toxicity Information System (CETIS). Tidepool Scientific Software, McKinleyville, CA 95519.
- HDR | HydroQual. 2013. Estimate of the BLM adjustment to the Nickel criterion for the Sanitary District of Decatur, Illinois. Prepared by Robert Santore (HDR | HydroQual).
- Oregon State University Aquatic Toxicology Laboratory (OSU). 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. June 2016.
- Toxicity Relationship Analysis Program (TRAP). U.S. Environmental Protection Agency. National Health and Environmental Effects Research Laboratory, Duluth, MN.
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- 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 Waters to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013.

Electronic Filing: Received, Clerk's Office 4/20/2018

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

APPENDIX A

Protocol

OSU Aquatic Toxicology Laboratory: Received, Clerk's Office 4/20/2018
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 ($\text{NiCl}_2 \times 6\text{H}_2\text{O}$; 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 ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 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 $\geq 60\%$ 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].

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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: William Stubblefield

Title: PROFESSOR

Date: 22 Nov 2016

Study Director:

Print Name: Allison Cardwell

Signature: Allison Cardwell

Title: Senior Faculty Research Assistant

Date: 22 November 2016

SUBJECT: SOP/PROTOCOL DEVIATION LOGProject and Test Nos.: Nickel Simulated Effluent with and without DOCDate of Occurrence: 24 December 2016Recorded 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 with 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 1 st and 2 nd broods (Days 4 and 5), but no 3 rd 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 re-conducted 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 re-conducted 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): <i>Allison Cardwell</i> Date: <i>1/9/2017</i>

Electronic Filing: Received, Clerk's Office 4/20/2018

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

APPENDIX B

Water Quality Summaries

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Electronic Filing: Received, Clerk's Office 4/20/2018

Water Effect Ratio (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia*, in a simulated effluent

Test #: Ni WER 1132R CDC

Water Quality Summary

TEST DAY	VHW RW (Concurrent)						DILUTED SIMULATED EFFLUENT/LAB WATER						2.1						2.9					
	NEW						NEW						NEW						NEW					
	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

TEST DAY	VHW RW (Concurrent)						DILUTED SIMULATED EFFLUENT/LAB WATER						2.1						2.9					
	OLD						OLD						OLD						OLD					
	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

Electronic Filing: Received, Clerk's Office 4/20/2018

Water Effect Ratio (WER) Testing: Chronic Toxicity of Nickel to the cladoceran, *Ceriodaphnia dubia*, in a simulated effluent

Test #: Ni WER 1132R CDC

Water Quality Summary

TEST DAY	4.2 NEW						6 NEW						8.5 NEW						12.2 NEW					
	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

TEST DAY	4.2 OLD						6 OLD						8.5 OLD						12.2 OLD					
	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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Electronic Filing: Received, Clerk's Office 4/20/2018

Water Effect Ratio (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)

TEST DAY	VHW RW (Concurrent)						DILUTED SIMULATED EFFLUENT/LAB WATER WITH DOC						4.5						6.5					
	NEW						NEW						NEW						NEW					
	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

TEST DAY	VHW RW (Concurrent)						DILUTED SIMULATED EFFLUENT/LAB WATER WITH DOC						4.5						6.5					
	OLD						OLD						OLD						OLD					
	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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

TEST DAY	9.2						13.2						18.9					
	NEW						NEW						NEW					
	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

TEST DAY	9.2						13.2						18.9					
	OLD						OLD						OLD					
	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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

TEST DAY	26.9						38.5					
	NEW						NEW					
	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

TEST DAY	26.9						38.5					
	OLD						OLD					
	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:

A handwritten signature in blue ink that reads 'Emily Biboux'.

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

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



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



ASL Report #: Q3850

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		Lab Information	
Client Sample ID: Cu 1124_#1 new		Lab Sample ID: Q385001	
Project Name: OSU Aquatic Toxicology Lab		Date Received: 12/27/16	
Sample Date: 12/15/16		Report Revision No: 0	
Sample Time: 17:00			
Type: Grab			
Matrix: Water			

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

CH2M ASL

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

CH2M ASL

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

CH2M ASL

Client Information		Lab Information	
Client Sample ID: Ni 1126_#1 new		Lab Sample ID: Q385011	
Project Name: OSU Aquatic Toxicology Lab		Date Received: 12/27/16	
Sample Date: 12/19/16		Report Revision No: 0	
Sample Time: 14:45			
Type: Grab			
Matrix: Water			

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		Lab Information	
Client Sample ID: Ni 1125_#1 new		Lab Sample ID: Q385012	
Project Name: OSU Aquatic Toxicology Lab		Date Received: 12/27/16	
Sample Date: 12/19/16		Report Revision No: 0	
Sample Time: 13:50			
Type: Grab			
Matrix: Water			

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

CH2M ASL

Client Information	Lab Information
Project Name: OSU Aquatic Toxicology Lab	Method Blank ID: WB1-1230
Sample Date: N/A	Date Received: N/A
Sample Time: N/A	Report Revision No: 0
Type: QC	
Matrix: Water	

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	Lab Information
Project Name: OSU Aquatic Toxicology Lab Type: QC Matrix: Water	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		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	DL	Chloride 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

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: 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	DL	RL	Sulfate Result	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

CH2M ASL

Client Information	Lab Information
Project Name: OSU Aquatic Toxicology Lab Type: QC Matrix: Water	Lab Batch ID: Q3850 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

CH2M ASL

Client Information		Lab Information	
Project Name: OSU Aquatic Toxicology Lab		Lab Batch ID: Q3850	
Type: QC		Report Revision No.: 0	
Matrix: Water			

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

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

Chain of Custody Record

COC #1

of 2
 1100 NE Circle Blvd. Suite 300
 Corvallis, OR 97330
 (541) 768-3120

Client Contact		Analysis Turnaround Time		Preservation Used		For Lab Use Only:	
Project Name: OSU - Dec. 2016 #2		TAT is Calendar days		4 1 1		SDG: 03850	
Project # or PO #: -		TAT if different from below:		Analysis Requested		Custody Seals Intact? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
Company Name: OSU Aquatic Tox Lab		<input type="checkbox"/> 14 days * <input checked="" type="checkbox"/> 21 days (STD) <input type="checkbox"/> 3 day *		Ca, Mg, Na, K		Hand delivered? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
Address: 33972 Texas St. SW		<input type="checkbox"/> 7 days * <input type="checkbox"/> 2 days *		Cl, SO ₄		Cooler Temp 64 °C	
City/State/Zip: Albany, OR 97321		<input type="checkbox"/> 5 days * <input type="checkbox"/> 1 day *		DOC		Therm ID No.: 123 Therm Exp.: 11/17	
Project Manager: Allison Cardwell		* (Surcharges will apply)		X		Packing Material: Circle Below	
Phone #: 541-926-1254		Sample Date		X		Ice (Blue Ice Box Bubble Wrap)	
Report to email: allison.cardwell@oregonstate.edu		Sample Time		X		Radiological Screen? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
Sample Identification (Limit of 20 characters)	Sample Date	Sample Time	Sample Type (C=Comp, G=Grab)	Matrix (Water, Soil, Air)	Total # of Cont.	Sample Specific Notes:	Lab ID:
Cu 1124 - #1 new	12/15/16	1700	G	H ₂ O	3		1
Cu 1124 - #6 new	12/15/16	1705	G	H ₂ O	3		2
Cu 1124 - #1 new 1	12/22/16	1400	G	H ₂ O	3		3
Cu 1124 - #1 - old	12/22/16	1045	G	H ₂ O	1		4
Cu 1124 - #2 - old	12/22/16	1050	G	H ₂ O	1		5
Cu 1124 - #3 - old	12/22/16	1055	G	H ₂ O	1		6
Cu 1124 - #4 - old	12/22/16	1100	G	H ₂ O	1		7
Cu 1124 - #5 - old	12/22/16	1110	G	H ₂ O	1		8
Cu 1124 - #6 - old	12/22/16	1115	G	H ₂ O	1		9
Cu 1124 - #7 - old	12/22/16	1040	G	H ₂ O	1		10
Ni 1126 - #1 new	12/19/16	1445	G	H ₂ O	3		11
Ni 1125 - #1 new	12/19/16	1350	G	H ₂ O	3		12
Ni 1126 - #7 new	12/21/16	1430	G	H ₂ O	1		13

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

Possible Hazard Identification: Yes No
 Are samples hazardous? Listed Ignitable Corrosive Reactive Toxic

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.

Relinquished by: Allison Cardwell Allison Cardwell 12/27/16 @ 1330
 Date/Time: 12/27/16 1701

Relinquished by: *Shirley Loggins*
 Date/Time: 12/27/16 1700

Shipped Via: UPS Fed-Ex USPS other
 Tracking #: _____

Return to Client Disposal by Lab Archive for _____ months

Received in Laboratory by: *Ram Bell*
 Special Instructions/QC Requirements
 If any samples are outside of holding time or temperature → proceed with analysis

COC # 2 of 2

Chain of Custody Record

Sample Identification (Limit of 20 characters)	Sample Date	Sample Time	Sample Type (C=comp, G=grab)	Matrix (Water, Soil, Air)	Total # of Cont.	Analysis Turnaround Time		Preservation Used		For Lab Use Only: SDG: Q3850 Custody Seals Intact? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No Hand delivered? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No Cooler Temp: 4°C Therm ID No: 123 Therm Exp: 12/17 Packing Material: Circle Below Ice (Blue Ice Box Bubble Wrap) Radiological Screen? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Sample Specific Notes:	Lab ID:
						TAT is Calendar days	TAT if different from below	Analysis Requested	Preservation Used			
Ni 1126-#8 new	12/21/16	1435	G	H ₂ O	1	<input checked="" type="checkbox"/>	21 days (STD)					14
Ni 1126-#7 old	12/22/16	1500	G	H ₂ O	1	<input checked="" type="checkbox"/>						15
Ni 1126-#8 old	12/22/16	1505	G	H ₂ O	1	<input checked="" type="checkbox"/>						110

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.

Sampled By: Allison Cardwell Allison Cardwell
 Received By: Ratal Bell
 Date/Time: 12/27/16 @ 1330
 Date/Time: 12/27/16 1401

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

Relinquished by: Allison Cardwell
 Relinquished by: Allison Cardwell
 Date/Time: 12/27/16 1400
 Date/Time: 12/27/16 1400

Shipped Via: UPS Fed-Ex USPS Other
 Tracking #: _____

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

A handwritten signature in black ink that reads '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.

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



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



ASL Report #: R1113

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

CH2M ASL

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

CH2M ASL

Client Information				Lab Information			
Client Sample ID: Paulina_1 filt				Lab Sample ID: R111306			
Project Name: OSU Aquatic Toxicology Lab				Date Received: 01/19/17			
Sample Date: 01/19/17				Report Revision No: 0			
Sample Time: 09:00							
Type: Grab							
Matrix: Water							

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

CH2M ASL

Client Information	Lab Information
Client Sample ID: Paulina_1 filt-PP	Lab Sample ID: R111307
Project Name: OSU Aquatic Toxicology Lab	Date Received: 01/19/17
Sample Date: 01/19/17	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									
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	Lab Information
Project Name: OSU Aquatic Toxicology Lab	Method Blank ID: WB1-0120
Sample Date: N/A	Date Received: N/A
Sample Time: N/A	Report Revision No: 0
Type: QC	
Matrix: Water	

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

CH2M ASL

Client Information	Lab Information
Project Name: OSU Aquatic Toxicology Lab	Blank Spike ID: BS1W0120
Type: QC	Report Revision No: 0
Matrix: Water	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

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	Chloride 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
 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: 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	RL	Sulfate 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

CH2M ASL

Client Information	Lab Information
Project Name: OSU Aquatic Toxicology Lab Type: QC Matrix: Water	Lab Batch ID: R1113 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

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.

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: 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	DL	Dissolved Organic Carbon 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

CH2M ASL

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

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):
SM5210B

CH2M ASL

Client Information				Lab Information			
Project Name: OSU Aquatic Toxicology Lab				Lab Batch ID: R1113			
Date Received: 01/19/17				Analysis Method: SM5210B			
Type: See C.O.C.				Units: mg/L			
Matrix: Water				Report Revision No.: 0			

Client Sample ID	Lab Sample ID	Dilution Factor	DL	RL	BOD5	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

CH2M ASL

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

Chain of Custody Record

Electronic Filing: Received, Clerk's Office 4/20/2018

Sample Identification (Limit of 20 characters)	Sample Date	Sample Time	Sample Type (C=Comp, G=Grab)	Matrix (Water, Soil, Air)	Total # of Cont.	Preservation Used														
						4	1	1	4	4	1	1	1							
Ni 1132R #1 new	1/12/17	1000	G	H ₂ O	3	X														
Ni 1132R #8 new	1/12/17	1005	G	H ₂ O	1															
Ni 1132R #1 old	1/13/17	1100	G	H ₂ O	1															
Ni 1132R #8 old	1/13/17	1105	G	H ₂ O	1															
Paulina - LM 1/2	1/18/17	1200	G	H ₂ O	3	X														
Paulina - 1 filt	1/19/17	0900	G	H ₂ O	3	X														
Paulina - 1 filt - PP	1/19/17	1200	G	H ₂ O	1															
OSU Effluent	1/19/17	1215	G	H ₂ O	1															

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 Calendar days
 14 days * 7 days * 5 days * 3 day * 2 days * 1 day *
 * (Surcharges will apply)

For Lab Use Only:
 SDG: R113
 Custody Seals Intact? Yes No
 Hand delivered? Yes No
 Cooler Temp 5.9 °C
 Therm ID No. 113 Therm Exp. 1/19/17
 Packing Material: Circle Below
 Plus Ice Box Bubble Wrap
 Radiological Screen? Yes No

Sample Specific Notes:
 Lab ID: 1
 2
 3
 4
 5
 6
 7
 8

Preservation Used: 1=Ice, 2=H2SO4, 3=HNO3, 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

Relinquished by: Allison Cardwell Allison Cardwell 1/19/17 @ 1330 Date/Time:
 Relinquished by: Emily Stefanson 1/19/17 @ 1420 Date/Time:
 Shipped Via: UPS Fed-Ex USPS Other Tracking #: _____

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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

APPENDIX C

Metals Data Summaries

Electronic Filing: Received, Clerk's Office 4/20/2018



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 ·6H ₂ O) (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	



Phillipsburg, NJ 9001.2006, 14001.2004
Paris, KY 9001.2008
Mexico City, Mexico 9001.2008
Deventer, The Netherlands 9001.2008, 14001.2004, 13485.2003
Selangor, Malaysia 9001.2008
Panaji, India 9001.2008
Gawice, Poland 9001.2008, 17025.2005

Richard M. Siberaki
Richard M. Siberaki
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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Nominal Conc. µg/L Ni	TOTAL CONC. µg/L Ni					NEW WATERS		OLD WATERS		ALL TOTAL	
	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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Nominal Conc. µg/L Ni	DISSOLVED CONC. µg/L Ni					NEW WATERS		OLD WATERS		ALL DISSOLVED	
	Day 0 New	Day 3 New	Day 4 Old	Day 6 New	Day 7 Old	Average Dissolved	Std Dev Dissolved	Average Dissolved	Std Dev Dissolved	Average Dissolved	Std Dev Dissolved
	12/16/2016	12/19/2016	12/20/2016	12/22/2016	12/23/2016	Conc. µg/L Ni	Conc. µg/L Ni	Conc. µg/L Ni	Conc. µg/L Ni	Conc. µg/L Ni	Conc. µg/L Ni
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

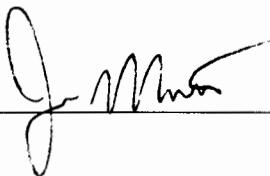
Method Blanks were not analyzed due to error in sample custody. The method blank samples were part of the concurrent non-DOC test which was re-tested and therefore the original samples for that test were not analyzed.

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

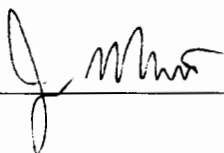
ASC 1/6/17

Electronic Filing: Received, Clerk's Office 4/20/2018

20170105_NiWer_1126.xlsx

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

Dilution Factor	Dilution Uncertainty	Sample ID	Instrument Data			Calculated Raw Data					Corrected For Dilution		Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertain y	% Error	% Recovery	
			Instrument avg. counts (Ni)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg	Calculated [Ni] (ug/L)	Uncertain y						Nominal (ug/L)
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 CDCT 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	

Jan 1/6/17

ASC 1/6/17

Electronic Filing: Received, Clerk's Office 4/20/2018

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)

Nominal Conc. µg/L Ni	TOTAL CONC. µg/L Ni					NEW WATERS			OLD WATERS			ALL TOTAL		
	Day 0 New	Day 3 New	Day 4 Old	Day 6 New	Day 7 Old	Average Total Conc. µg/L Ni	Std Dev Conc. µg/L Ni	Total µg/L Ni	Average Total Conc. µg/L Ni	Std Dev Conc. µg/L Ni	Total µg/L Ni	Average Total Conc. µg/L Ni	Std Dev Conc. µg/L Ni	Total 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

Electronic Filing: Received, Clerk's Office 4/20/2018

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)

Nominal Conc. µg/L Ni	DISSOLVED CONC. µg/L Ni					NEW WATERS		OLD WATERS		ALL DISSOLVED	
	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

Electronic Filing: Received, Clerk's Office 4/20/2018

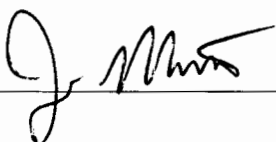
20170207_NiWer_1132R.xlsx

1 of 2

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: _____

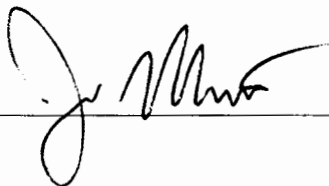
2/9/17

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 ug/L Ni in the old sample the next day. 2/9/17 ASC

Analyst:



Date:

2/9/17

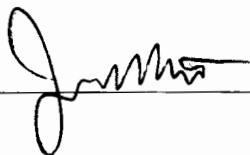
ASC 2/10/17

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 (µg/L Ni)	Measured [Ni] (µg/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	

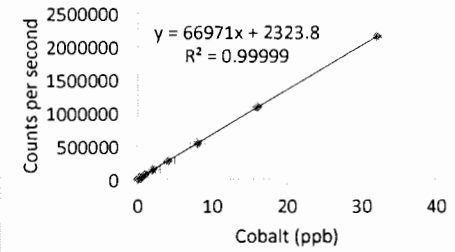
Analyst: _____



Date: 2/2/17

ASC 2/10/17

Date of Run	Linear Dynamic Range (LDR)	$\sum(x_i)^2$	$\sum(x_i)$	D	n	Sample Reqs	Detection Limit (ppb)	1% HNO3	Slope	Intercept	Intercept Stdev	
2/7/17	32	1.37E+03	64	3.28E+04	27	3.0	0.008	Slope Stdev	66971	2324	920	
Standard Uncertainty								Limit of Quantization	R ²	0.99999	2258	Stdev (Y)
		5.0%	0.8%									



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%

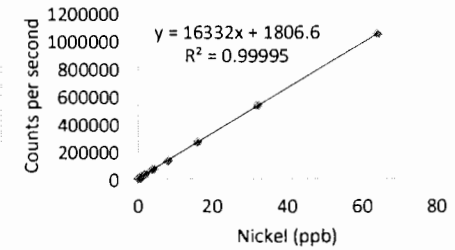
Dilution Factor	Dilution Uncertainty	Sample ID	Instrument Data			Calculated Raw Data			Corrected For Dilution			Instrument [Co] ug/L	Calculated [Co] (ug/L)	Uncertainty y	% Error	% Recovery		
			Instrument avg. counts (Co)	Stdev	Rstdev	Instrument In (%)	In Stdev	Instrument Calculated [Co] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated (ug/L)						Uncertainty	Nominal (ug/L)
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 Blank	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	0.0	BMDL	-	-	-
1		OSU Effluent 01/12/17 D	2222	88	4%	77.70%	0.21%	0.03	0.00	2222	BMDL	-	0	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	0.2	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	1.1	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	1.0	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	1.1	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	1.1	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	1.1	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	1.1	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	1.1	1.08	0.02	-	-
1		Blank	-63	61	96%	99.25%	0.90%	0.00	0.00	-63	BMDL	-	0	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	1.1	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	1.1	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	0.9	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	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	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	0.9	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	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	1.0	0.94	0.02	-	-

(Signature) 2/9/17

ASC 2/10/17

Date of Run	Linear Dynamic Range (LDR)	$\Sigma(x_i)^2$	$\Sigma(x_i)$	D	n	Sample Reqs	Detection Limit (ppb)	1% HNO3	Slope	Intercept	Intercept Stdev
2/7/17	64	5.46E+03	128	1.31E+05	27	3.0	0.029	Slope Stdev	16332	1807	1068
		x	y				Limit of Quantization	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%

Dilution Factor	Dilution Uncertainty	Sample ID	Instrument Data				Calculated Raw Data				Corrected For Dilution							
			Instrument avg. counts (Ni)	Stdev	Rstdev	In (%)	Instrument Calculated [Ni] (ppb)	Instrument Stdev	Blank Corrected Avg.	Calculated [Ni] (ug/L)	Uncertainty y	Nominal (ug/L)	Instrument [Ni] ug/L	Calculated [Ni] (ug/L)	Uncertainty y	% Error	% Recovery	
1		Standard 0	0	23	0%	100.00%	0.00	0.00	0.00	0	BMDL	-	0	BMDL	BMDL	-		
1		Standard 1	8933	152	2%	90.97%	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	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	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	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	7.92	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	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	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	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	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	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	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	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	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	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	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	0.13			
1		Blank	2332	93	4%	99.25%	0.90%	0.14	0.01	2332	0.0	0.1	0	0.1	0.10			
1		QC Standard	183737	1516	1%	95.44%	0.47%	11.05	0.09	183737	11.1	0.1	10	11.1	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	0.10	4.8%	104.8%	
1		Ni WER 1132R CDC D 11164 +5.	239566	831	0%	84.04%	0.56%	14.41	0.05	239566	14.6	0.1	12.9	14.4	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	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	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	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	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	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	0.13			

2/9/17

ASC 2/10/17

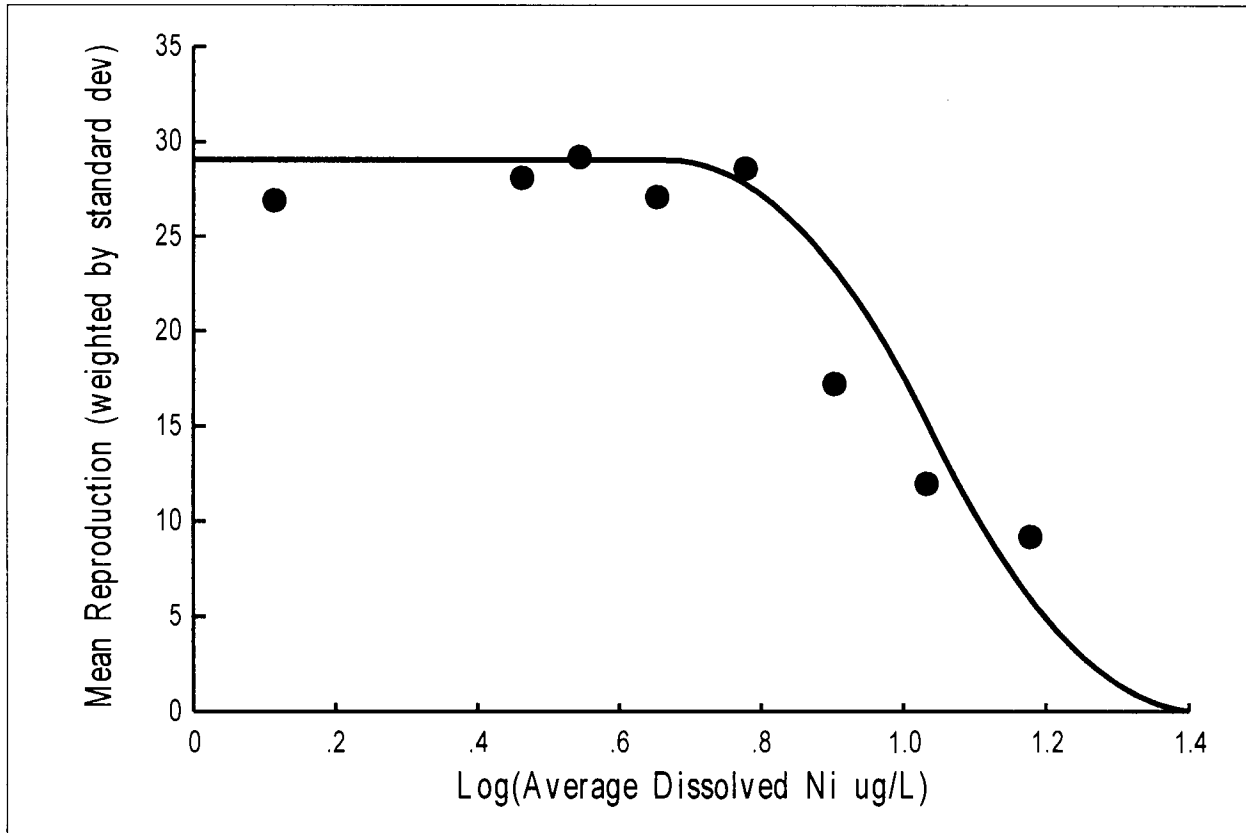
Electronic Filing: Received, Clerk's Office 4/20/2018

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

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
LogX 50	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

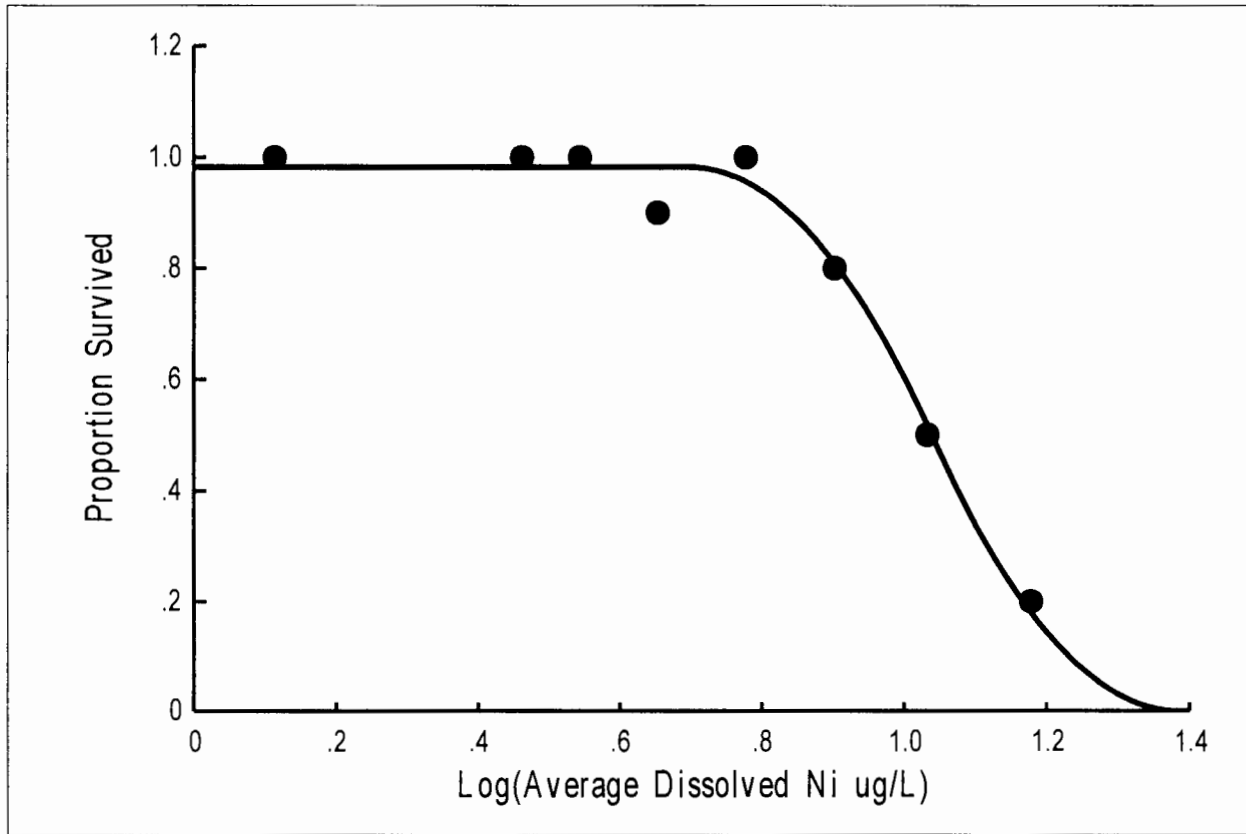
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.1139	26.9000	29.0512	2.1512	6.7900
0.4624	28.1000	29.0512	0.9512	5.5470
0.5441	29.2000	29.0512	-0.1488	5.5340
0.6532	27.1000	29.0512	1.9512	9.0120
0.7782	28.6000	27.7737	-0.8263	2.2210
0.9031	17.2000	23.3148	6.1148	10.9000
1.0334	12.0000	15.2535	3.2535	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
LogX 50	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)

CETIS Summary Report

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

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

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%

CETIS Summary Report

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

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

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%

CETIS Analytical Report

Report Date: 28 Mar-17 12:23 (p 2 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

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

CETIS Analytical Report

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

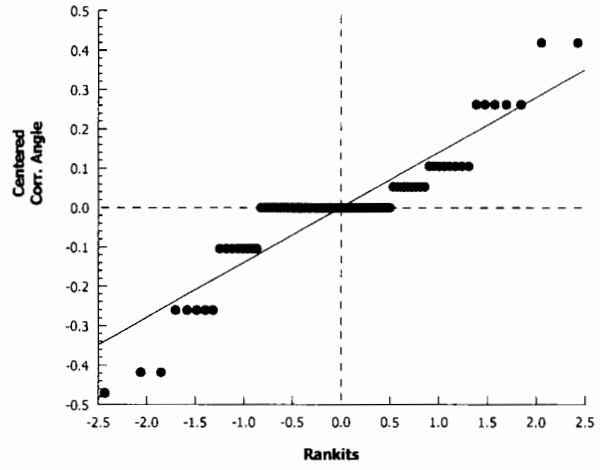
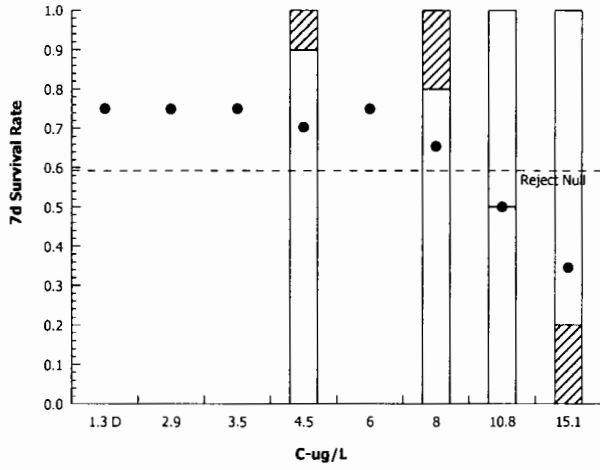
Ceriodaphnia 7-d Survival and Reproduction Test

OSU Aquatic Tox Lab

Analysis ID: 12-8224-6664 Endpoint: 7d Survival Rate
 Analyzed: 28 Mar-17 12:22 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics



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%

CETIS Analytical Report

Report Date: 28 Mar-17 12:24 (p 2 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

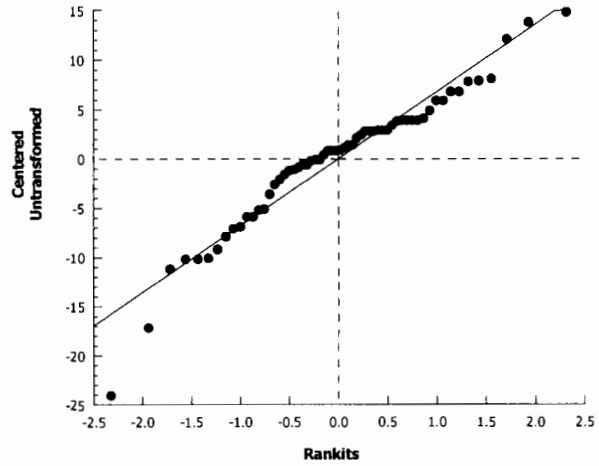
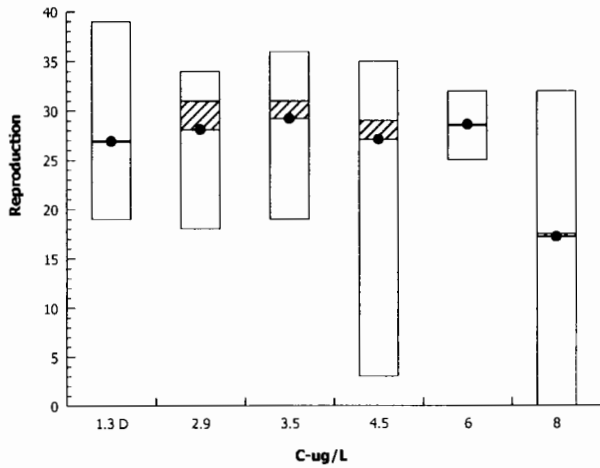
Analysis: Nonparametric-Control vs Treatments

Official Results: Yes

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



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 x 6H ₂ O)	Species: <i>Ceriodaphnia dubia</i>
Dilution Water: Diluted Simulated Effluent RW (Reconstituted Lab H ₂ O) (w/B ₁₂ and Se) (at a 20% dilution from full Simulated)	Organism Lot or Batch Number: 010917 VHW 010917 Sim Eff
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 TRC: Initiation TDS: Daily
Test Conc.: 0 (Very Hard RW Conc.), 0 (Simulated RW no DOC/Control Dilution H ₂ O), 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 monobords 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 x 6H ₂ O		
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		
	1/10/17		
	1/12/17		

PREPARATION OF TEST SOLUTIONS

Nominal Chemical Conc. (µg/L Ni)	Nominal Stock Volume (µL)	Test Stock Volume (µL)		
		Day 0	Day 2 ⁽²⁾	Day 3 ⁽³⁾
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	1/10/17	1/12/17
Time		1030	0950	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: B₁₂ and Se

(Stock: Vitamin B₁₂ 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B₁₂)

(Stock: Na₂SeO₄ 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.

OTH 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)

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 x 6H ₂ O		
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	Day 3	Day 4	Day 5
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: B₁₂ and Se

(Stock: Vitamin B₁₂ 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B₁₂)

(Stock: Na₂SeO₄ 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 x 6H ₂ O		
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	1/13/17		
	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: B₁₂ and Se

(Stock: Vitamin B₁₂ 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B₁₂)

(Stock: Na₂SeO₄ 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

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1132R CDC - no DOC										
Test Species: <i>C. dubia</i>										
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)	487	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	8.8			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2350	2310	2300	2260	2230	2290	2320			
TDS (mg/L)	1185	1165	1161	1139	1125	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									
Alk. (mg/L)	432									
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 NH₃ 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

①-TH 1/9/17 WP ②-TH 1/15/17 E

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1132R CDC - no DOC										
Test Species: <i>C. dubia</i>										
Conc. ($\mu\text{g/L Ni}$)	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. ($^{\circ}\text{C}$)	25	25	25	25	25	25	25			
Cond. ($\mu\text{S/cm}$)	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. ($^{\circ}\text{C}$)	25	25	25	25	25	25	25			
Cond. ($\mu\text{S/cm}$)	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. ($^{\circ}\text{C}$)	25	25	25	25	25	25	25			
Cond. ($\mu\text{S/cm}$)	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:	12:40	1250	1325	1300	1300	1300	1305			
Initials:	TH	TH	TH	MS	TH	TU	TH			

NOTE: Hardness, alkalinity, TRC, and NH₃ 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 $^{\circ}\text{C}$ prior to mixing the dilution series. The temperature of resulting dilutions is assumed to also be 25 $^{\circ}\text{C}$.

OTH 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: <i>C. dubia</i>										
Conc. ($\mu\text{g/L Ni}$)	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. ($^{\circ}\text{C}$)	25	25	25	25	25	25	25			
Cond. ($\mu\text{S/cm}$)	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. ($^{\circ}\text{C}$)	25	25	25	25	25	25	25			
Cond. ($\mu\text{S/cm}$)	2350	2320	2310	2290	2260	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. ($^{\circ}\text{C}$)	25	25	25	25	25	25	25			
Cond. ($\mu\text{S/cm}$)	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:	4/9/17	4/10/17	4/11/17	4/12/17	4/13/17	4/14/17	4/15/17			
Time:	1340	1250	1325	1300	1300	1300	1310			
Initials:	TH	TH	TH	M	TH	TH	TH			

NOTE: Hardness, alkalinity, TRC, and NH₃ 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

SUBJECT: DAILY TOXICITY TEST LOG

Project Number: N. WER 1132 R CDC

Test Species: C. dubia

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

date	Int	
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

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1132R CDC - no DOC										
Test Species: <i>C. dubia</i>										
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)	509	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	2270	2360	2350	2410			
TDS (mg/L)	1293	1186	1220	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	2370	2290	2400	2490	2440 2500			
TDS (mg/L)	1556	1252	1254	1158	1210	1260	1220 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	2290	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	8.89	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	1178	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)	2560	2390	2390	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			

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1132R CDC - no DOC										
Test Species: <i>C. dubia</i>										
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			

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	1/6	1/7	1/4	1/6	1/6	1/6	1/1	1/6	1/5			
	5	0	2/8	0	1/30	2/11	0	2/11	2/10	0	2/13			
	6	2/12	0	2/9	0	0	2/11	0	0	2/8	0			
	7	2/18	2/17	2/20	2/15	2/17	2/16	2/19	0	2/18	2/15			
	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	2/8	0	0	0			
	7	2/7	2/14	2/10	2/10	2/20	2/14	0	2/16	2/17	2/23			
	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	2/11	2/11	0	0			
	6	2/12	0	2/3	0	0	0	0	0	0	0			
	7	2/13	2/17	2/18	2/17	2/17	2/12	2/15	2/16	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:		1/17	1/17	1/17	1/17	1/17	1/17	1/17	1/17	X = Original organism has died.				
Time:		1335	1400	1315	1350	1245	1245	1410		M = Male.				
Initials:		TH	TH	MS	TH	TH	TH	TH						

OTH 1/14/17E @ TH 1/15/17E

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

SUBJECT: CERIODAPHНИЯ DUBIA CHRONIC BIOLOGICAL DATA

Project Number: Ni WER 1132R CDC - no DOC														
		Number of Neonates Produced and Survival of Original Organisms										Total	Mean	Remarks
Conc. $\mu\text{g/L Ni}$	Day	A	B	C	D	E	F	G	H	I	J			
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	2/17	2/17	2/14	2/16	2/14	2/15	2/15	2/16	2/15	2/15			
	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	1/12		2/9	0	2/12			
	6	2/10	0	0	0	2/11	0		0	2/11	0			
	7	2/10	2/13	2/19	2/16	2/13	2/14		2/13	2/11	2/15			
	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	1/10	1/10	2/10	2/8	1/8	2/8	2/9	0	2/11			
	6	2/11	0	0	0	0	0	0	0	2/11	0			
	7	2/10	2/16	2/15	2/16	2/13	2/13	2/13	2/16	2/11	2/13			
	8													
	Total		28	32	30	31	25	27	26	30	28	29		
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	1400	1250	1250	1420		M = Male.					
Initials:	TH	TH	TH	TH	TH	TH	TH							

TH 1/16/17 E

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
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	0X	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 2/7	↓	0	0	0	2/9	2/9	0	2/5			
	6	2/14	0	↓	2/4	2/12	2/4	0	0	2/3	0			
	7	2/12	2/11	↓	0	↓	0	2/11	0	2/14	2/6			
	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	0X	0	0	0	2/6	2/9	0	0			
	6	0	0	↓	2/9	0X	2/6 X	0	0	2/11	2/5 X			
	7	0	0	↓	0	↓	↓	0	2/3 X	2/11	↓			
	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	0X	1/5	1/3	1/5	1/5	1/6	1/6	1/5			
	5	0	2/4	↓	2/2	1/1 X	0X	2/6	2/8	0	0			
	6	0X	0X	↓	0	↓	↓	0X	0	2/9 X	2/5			
	7	↓	↓	↓	2/8	↓	↓	↓	2/4 X	↓	2/2			
	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	1/21/17	1/31/17	1/4/17	1/5/17	1/6/17	1/7/17	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: <i>C. dubia</i>		
General Comments	For Concurrent VHW: Neonates obtained from (culture): CD010817 on ^{VHW} 1/9/17 TH For Diluted Simulated Effluent RW: Neonates obtained from (culture): CD123016 ^{S.M.E.H.} 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 M)	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 1500 TH Old (comp) Total and Dissolved (0.45 µm) sampled at: 1545 TH	

@TH 1/16/17E

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

Electronic Filing: Received, Clerk's Office 4/20/2018

SIMULATED EFFLUENT FOR TESTING - *Ceriodaphnia dubia*

Total hardness = **400** mg/L as CaCO₃
 Alkalinity = **400** mg/L as CaCO₃
 Volume of water = **16** L (with 20% addition, will be total volume of 20-L)

Amount weighed		
45.330	4.53299	grams CaSO ₄ · 2H ₂ O
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

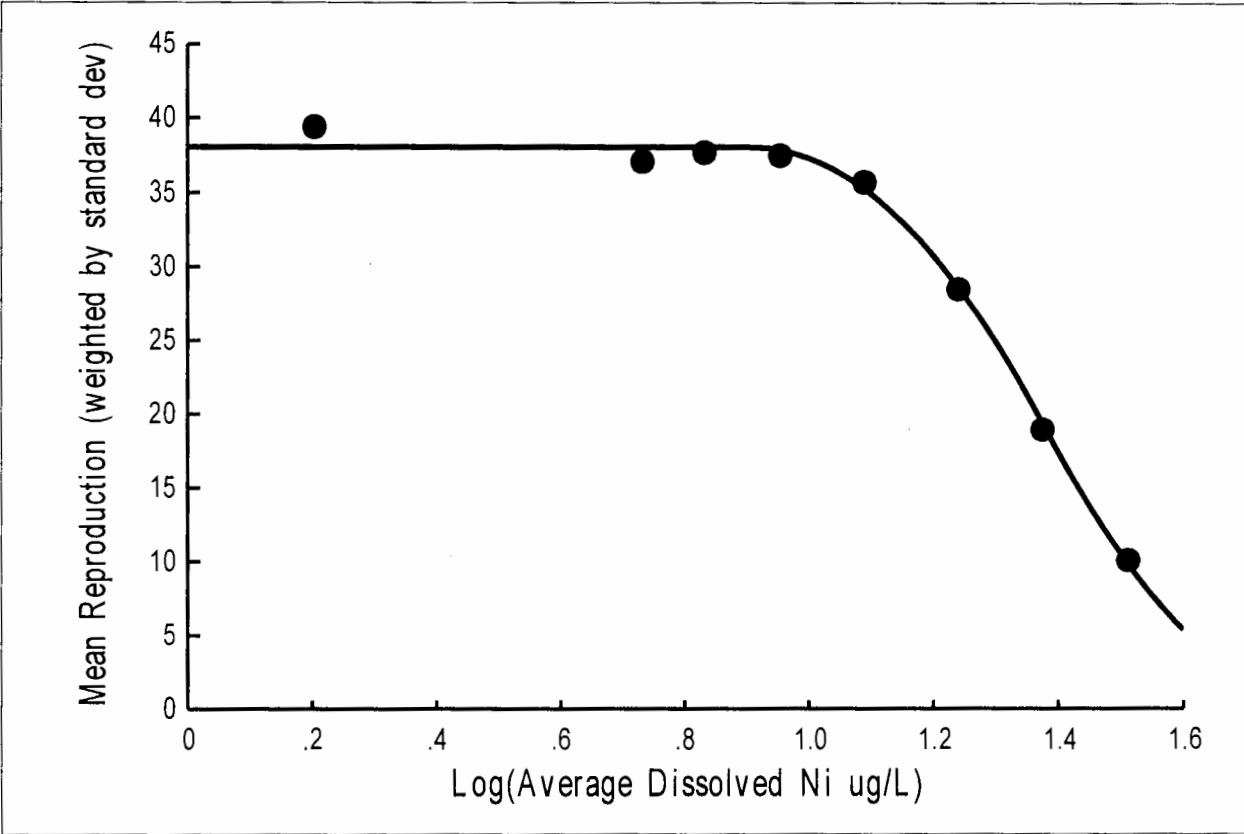
	Manufacturer	Lot #
CaSO ₄ · 2H ₂ O	ACROS	A0363568
MgSO ₄	EMD	151020001
KCl	Alfa Aesar	E294012
NaCl	JT Baker	0000157485
NaHCO ₃	Macron	0000134421

Recon. Water #: 1485
 Test #: 1132R
 Date/Time Prepared: 1/8/17 TH
 Technician: TH

PREPARATION STEPS:

- 1) In a gallon jar, add CaSO₄ · 2H₂O to 3-L DI. Put on stir plate. Mix overnight
- 1/6/17 TH { 2) In a gallon jar, add MgSO₄ to 3-L DI. Put on stir plate. Mix overnight
- 3) In 2-Liter Beaker, add KCl, NaCl, and NaHCO₃ 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
- 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".
- 1/8/17 TH { 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
LogX 50	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		

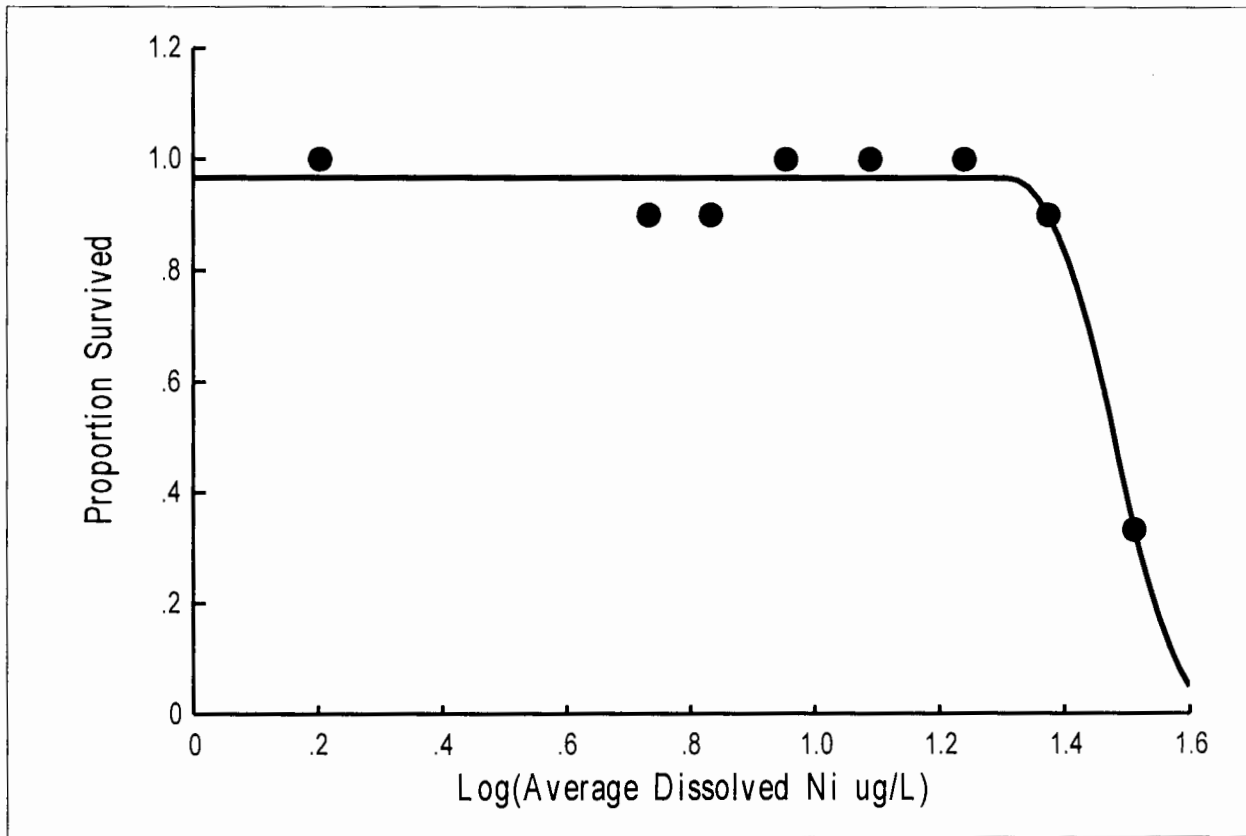
Data Summary

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

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%

CETIS Summary Report

Report Date: 24 Mar-17 12:57 (p 2 of 2)

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

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	

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	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 24 Mar-17 12:57	Analysis: STP 2x2 Contingency Tables	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	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	

Ceriodaphnia 7-d Survival and Reproduction Test

OSU Aquatic Tox Lab

Analysis ID: 09-6730-7280
 Analyzed: 24 Mar-17 12:57

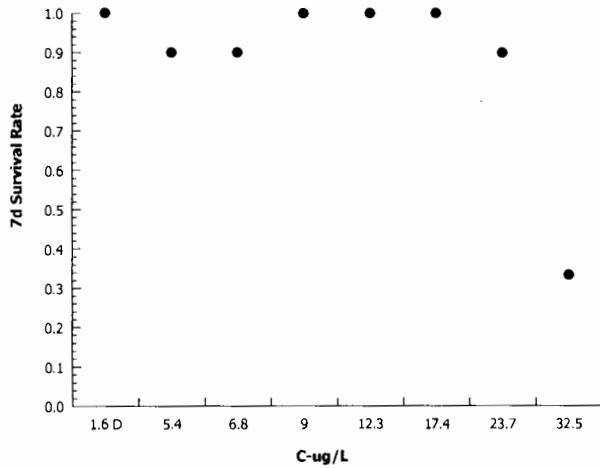
Endpoint: 7d Survival Rate
 Analysis: STP 2x2 Contingency Tables

CETIS Version: CETISv1.8.7
 Official Results: Yes

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



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 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	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%

Ceriodaphnia 7-d Survival and Reproduction Test

OSU Aquatic Tox Lab

Analysis ID: 17-7379-4994
 Analyzed: 24 Mar-17 12:57

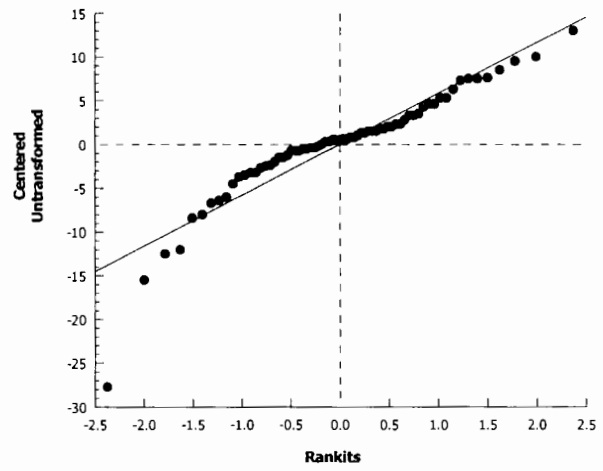
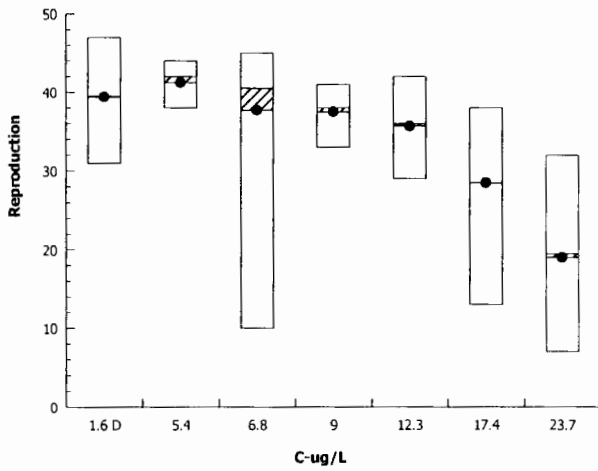
Endpoint: Reproduction
 Analysis: Nonparametric-Multiple Comparison

CETIS Version: CETISv1.8.7
 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



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%

CETIS Analytical Report

Report Date: 24 Mar-17 12:57 (p 4 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
 Analyzed: 24 Mar-17 12:57

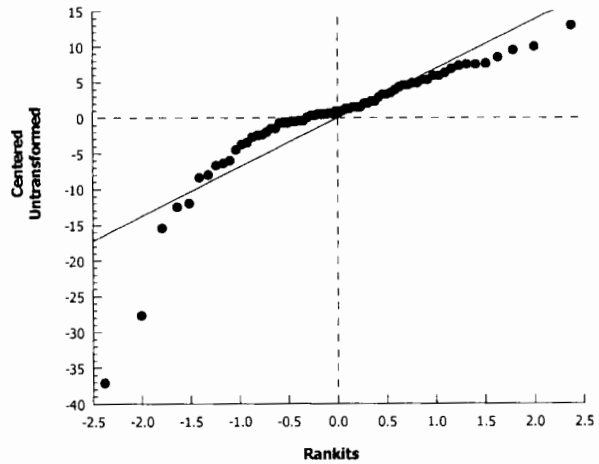
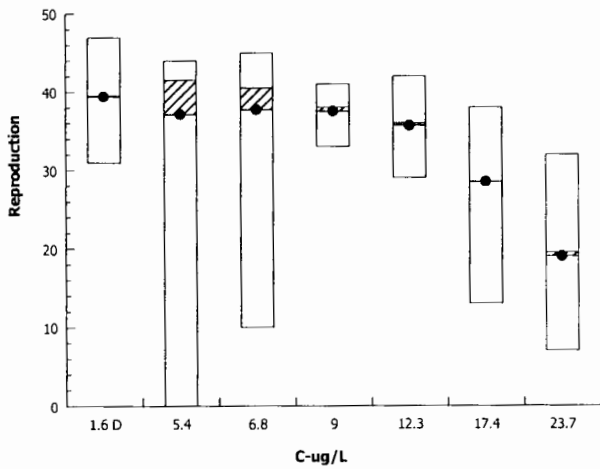
Endpoint: Reproduction
 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 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



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		

Electronic Filing: Received, Clerk's Office 4/20/2018

CETIS Test Data Worksheet

Report Date: 24 Mar-17 12:20 (p 2 of 3)

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		

CETIS Test Data Worksheet

Report Date: 24 Mar-17 12:20 (p 3 of 3)

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
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		

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 x 6H ₂ O)	Species: <i>Ceriodaphnia dubia</i>
Dilution Water: With DOC-Diluted Simulated Effluent RW (Reconstituted Lab H ₂ O) (w/B ₁₂ and Se)	Organism Lot or Batch Number: C-1 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 TRC: Initiation TDS: Daily
Test Conc.: 0 (Simulated RW WITH DOC/Control Dilution H ₂ O), 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 of 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 <u>3</u> 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 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).

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

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1126 CDC – WITH DOC

Chemical	Nickelous Chloride Hexahydrate NiCl ₂ x 6H ₂ O			
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 ID¹		Sim. RW with DOC RW * 1474-B	Sim. RW with DOC RW * 1474-B	Sim. RW with DOC RW * 1474-B			
Concurrent Control Water ID¹							
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: B₁₂ and Se

(Stock: Vitamin B₁₂ 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B₁₂)

(Stock: Na₂SeO₄ 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

SUBJECT: TEST SUBSTANCE USAGE LOG – CHEMICAL TESTING

Project Number: Ni WER 1126 CDC – WITH DOC

Chemical	Nickelous Chloride Hexahydrate NiCl ₂ x 6H ₂ O		
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: B₁₂ and Se(Stock: Vitamin B₁₂ 60 mg/L Stock = add 50 µL/L to achieve 3 µg/L B₁₂)(Stock: Na₂SeO₄ 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

©ASC 2/10/17 E

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1126 CDC - WITH DOC										
Test Species: <i>C. dubia</i>										
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	9.6		M07	
Temp. (°C)	25	24.25	25	25	25	25	25		dig	
Cond. (µS/cm)	2270	2320	2300	2330	2340	2330	2330		M03	
TDS (mg/L)	1145	1170	1169	1178	1184	1178	1191		M03	
Hard. (mg/L)	324			304			304		titr	
Alk. (mg/L)	392			400			408		titr	
TRC (mg/L)	20.09			NT			NT		PCII	
NH ₃ (mg/L)	21.0			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	9.5			
Temp. (°C)	25	25	25	25	25	25	25			
Cond. (µS/cm)	2270	2330	2320	2320	2340	2340	2330			
TDS (mg/L)	1149	1180	1172	1176	1183	1181	1191			
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	9.5			
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	12/19/16	12/19/16	12/20/16	12/21/16	12/22/16			NT = not taken
Time:	1500	1520	1540	1355	1550	1425	1335			
Initials:	TH	ES	ES	ASC	TH	ES	MS			

NOTE: Hardness, alkalinity, TRC, and NH₃ 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

① ES 12/17/16 E ② TH 12/20/16

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1126 CDC - WITH DOC										
Test Species: <i>C. dubia</i>										
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	1425	1425	1335			
Initials:	TH	ES	ES	ASC	TH	ES	M			

NOTE: Hardness, alkalinity, TRC, and NH₃ 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.

OT 12/20/16

ES 1/17/17

ASC 2/10/17

SUBJECT: CHRONIC CHEMICAL DATA (INITIAL)

Project Number: Ni WER 1126 CDC - WITH DOC										
Test Species: <i>C. dubia</i>										
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	M			

NOTE: Hardness, alkalinity, TRC, and NH₃ 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

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1126 CDC - WITH DOC										
Test Species: <i>C. dubia</i>										
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	4.5									
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	8.5	8.6		M07	
Cond. (µS/cm)	2370	2470	2370	2410	2570	2440	2440		M03	
TDS (mg/L)	1198	1243	1200	1221	1308	1237	2440.265		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	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	8.8	8.6			
Cond. (µS/cm)	2650	2600	2430	2550	2730	2660	2560			
TDS (mg/L)	1343	1321	1231	1300	1342	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	2440	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	M	M	TH			

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

SUBJECT: CHRONIC CHEMICAL DATA (FINAL)

Project Number: Ni WER 1126 CDC - WITH DOC										
Test Species: <i>C. dubia</i>										
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	1267	1293	1203	1326	1277	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	MI	MS	TH			

① ES 12/18/16 E ② TH 12/23/16 E ASC 2/10/17 ES 1/17/17

SUBJECT: CERIODAPHНИЯ 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	4/18	3/23	3/17	3/18	3/22	3/24	3/18	3/22	3/21	3/22			
	8	with 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	Key to Symbols:				
Date:		12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16		X = Original organism has died.				
Time:		1530	1600	1400	1530	1500	1420	1420		M = Male.				
Initials:		ES	ES	ES	ES	MS	ES	MS						

① MS 12/23/16 R

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

SUBJECT: CERIODAPHНИЯ 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	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	3/19	3/18	3/22	3/20	3/18	3/26	3/27	3/22			
	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	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	2/4	2/10			
	6	0	0	0	2/13	2/14	0	0	0	0	0			
	7	3/16	3/23	3/28	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	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	Key to Symbols:					
Date:	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16	X = Original organism has died.						
Time:	1530	1600	1400	1530	1500	1420	1420	M = Male.						
Initials:	ES	ES	ES	ES	M)	ES	M)							

① M 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
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 [ⓑ]	1/4	1/6 [ⓑ]	1/5	1/5 [ⓑ]	1/6	1/5	1/5	1/3 [ⓑ]	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	3/14	3/14	3/8	0	0X			
	8										↓			
	Total	11	20	17	32	19	21	29	21	7	13			
38.5	1	0	0	0	0X [ⓐ]	0	0	0	0	0	0			ⓐ org not found
#8	2	0	0	0		0	0X	0	0	0	0			12/17/16 ES
	3	0	0	0		0 [ⓑ]		0	0	0	0			
	4	1/4 [ⓑ]	1/4X	1/6 [ⓑ]		1/5 [ⓑ]		1/6	1/6	1/5 [ⓑ]	1/6			
	5	1/1 [ⓑ]		0 [ⓑ]		0 [ⓑ]		0	0	0 [ⓑ]	0			
	6	0X		2/2X		2/10		2/9	2/12	0X	2/6X			
	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	Key to Symbols:				
	Date:	12/17/16	12/18/16	12/19/16	12/20/16	12/21/16	12/22/16	12/23/16		X = Original organism has died.				
	Time:	1530	1600	1400	1530	1500	1420	1420		M = Male.				
	Initials:	ES	ES	ES	ES/MS	MS	ES	MS						

ⓐ TH 12/20/16 E

? MS 12/21/16 E

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

SUBJECT: DAILY TOXICITY TEST LOG

Project Number: Ni WER 1126 CDC – WITH DOC		
Test Species: <i>C. dubia</i>		
General Comments	For Diluted Simulated Effluent RW: Neonates obtained from (culture): CD 120816 on 12/16/16 MS S.M. Eff.	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 M 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 i-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

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SIMULATED EFFLUENT FOR TESTING - *Ceriodaphnia dubia*

Total hardness = **400** mg/L as CaCO₃
 Alkalinity = **400** mg/L as CaCO₃
 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 ₂ O
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 · 2H ₂ O	ACROS Organics	A0363568
MgSO ₄	EMD	151020001
KCl	Alfa Aesar	E294012
NaCl	EMD	YCO4E/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 CaSO₄ · 2H₂O to 3-L DI. Put on stir plate. Mix overnight
- ✓ 2) In 4-Liter Flask, add MgSO₄ to 3-L DI. Put on stir plate. Mix overnight
- ✓ 3) In 2-Liter Beaker, add KCl, NaCl, and NaHCO₃ 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

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Prepared by

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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.

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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

Nominal Treatment µg/L Ni	Measured (Average) Dissolved Ni µg/L	Rep	Survival	total # of neonates	Per original female		Per surviving female	
					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

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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

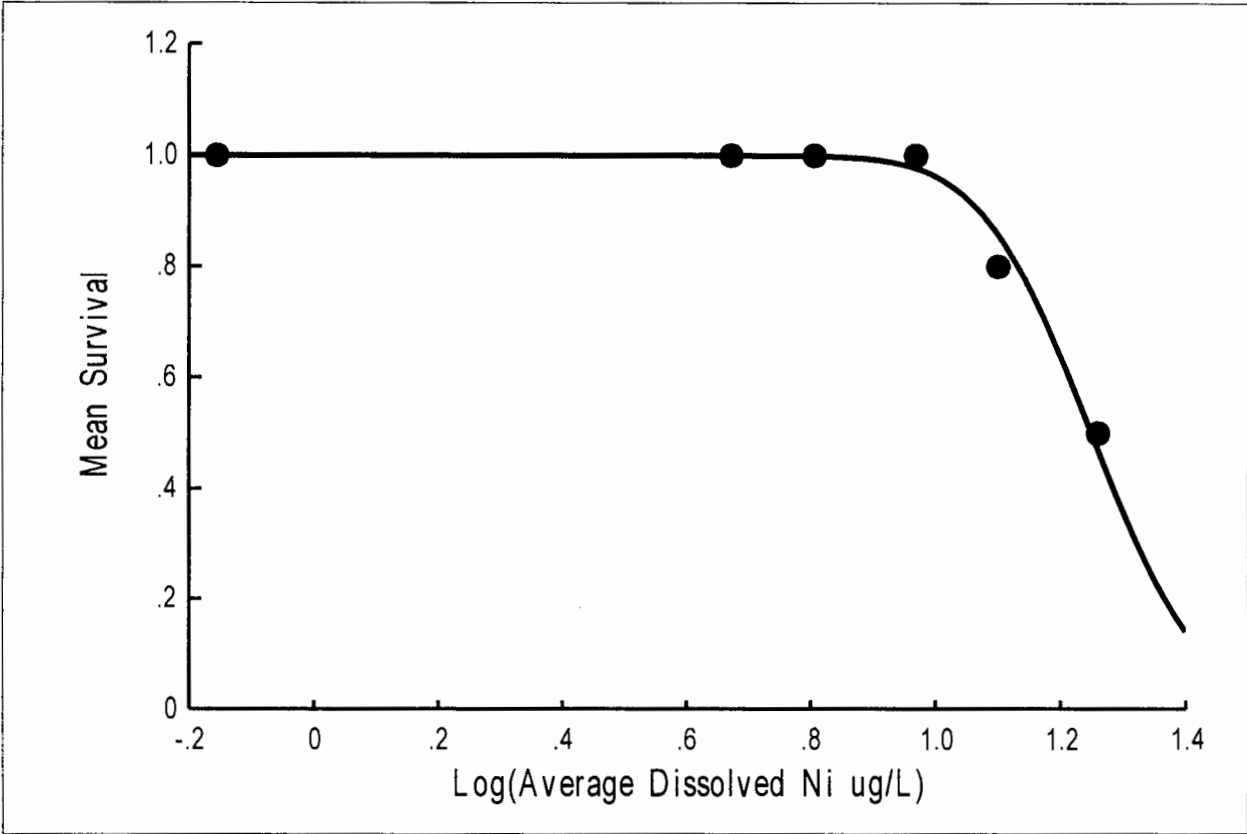
Nickel WER (simulated effluent tested concurrently with SDD effluent)

Test ID: Ni Sim 1008 CDC

Nominal Treatment µg/L Ni	Measured (Average) Dissolved Ni µg/L	Rep	Survival	total # of neonates	Per original female		Per surviving female	
					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
LogX 50	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

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

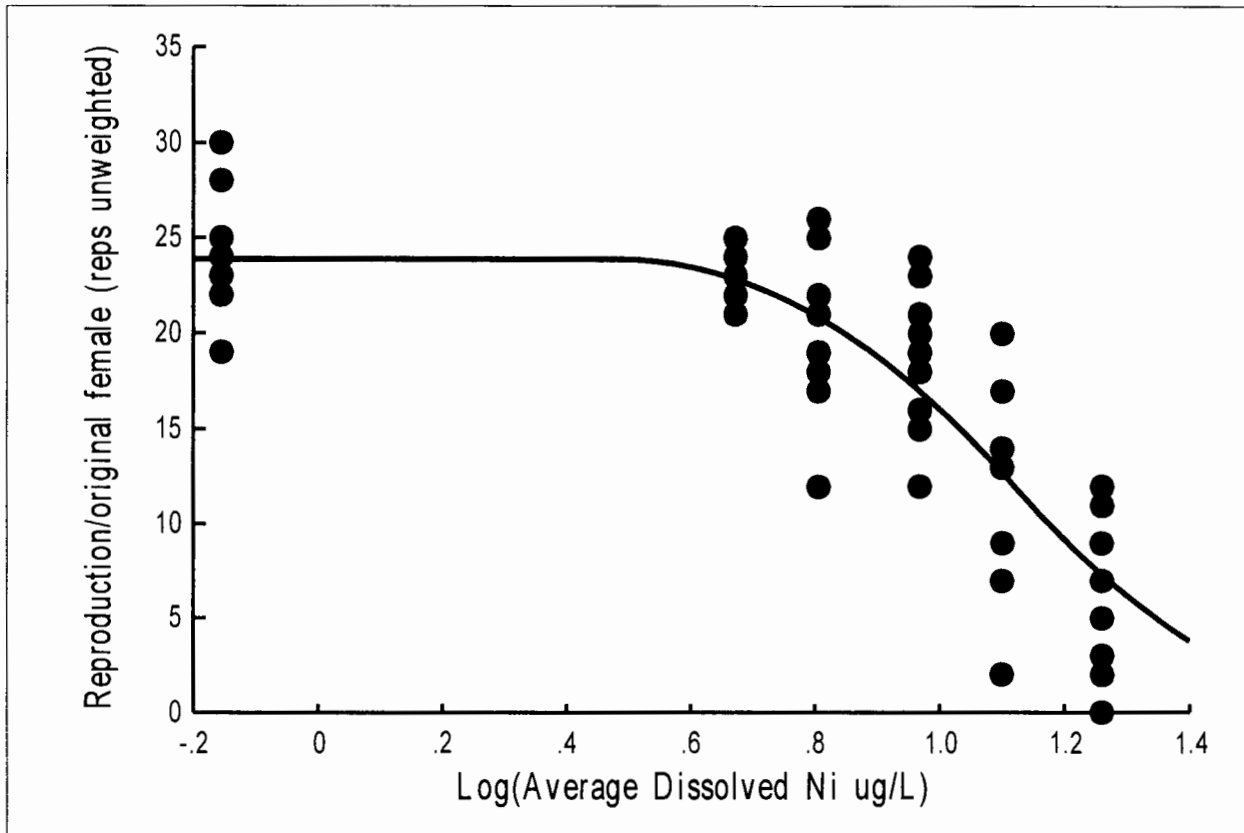
Data Summary

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

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



Parameter Summary (Threshold Sigmoid Regression Analysis)

Parameter	Guess	FinalEst	StdError	95%LCL	95%UCL
LogX 50	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

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.
0.6721	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.

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

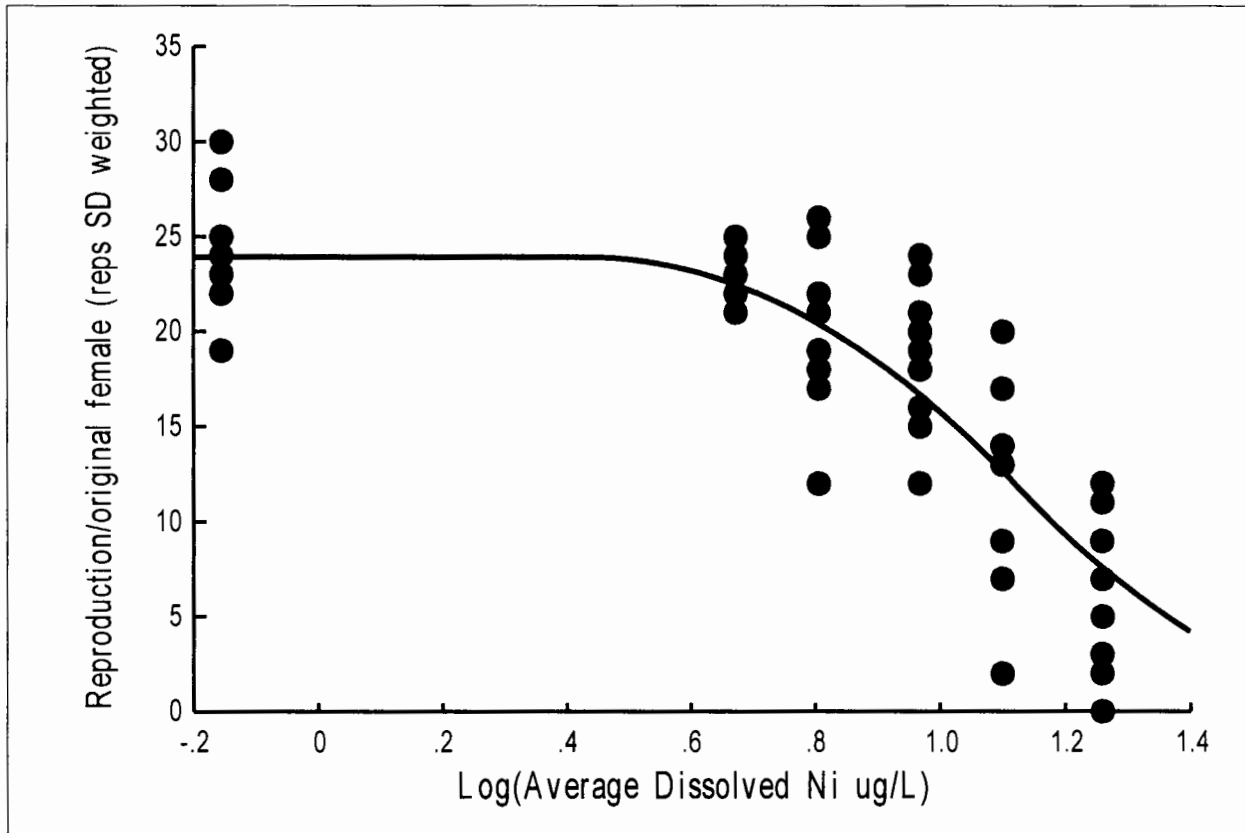
Data Summary				
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8062	18.0000	20.8452	2.8452	1.
0.8062	18.0000	20.8452	2.8452	1.
0.8062	17.0000	20.8452	3.8452	1.
0.8062	12.0000	20.8452	8.8452	1.
0.9685	24.0000	17.0079	-6.9921	1.
0.9685	23.0000	17.0079	-5.9921	1.
0.9685	21.0000	17.0079	-3.9921	1.
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.

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

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	1.1114	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

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
0.6721	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

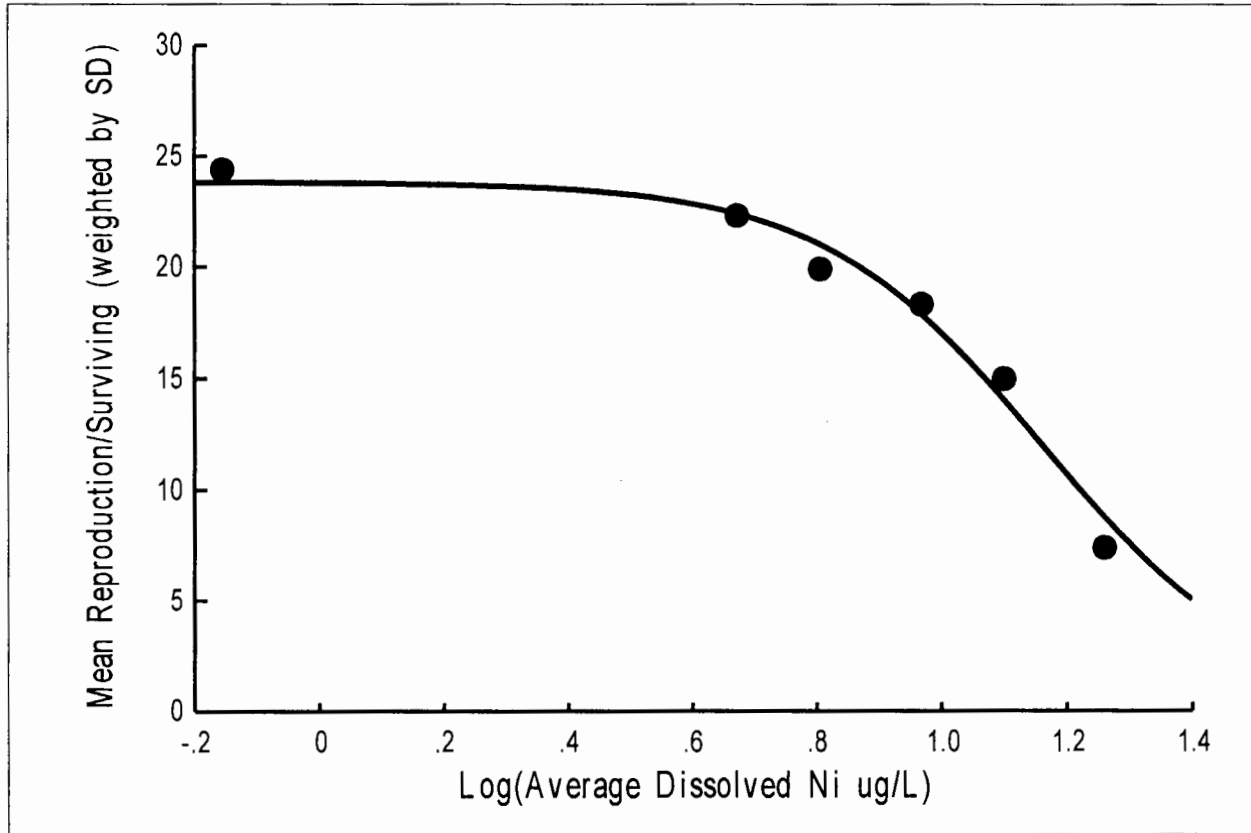
Expos Var	Obs Eff Var	Fit Eff Var	Residual	Weight
0.8062	18.0000	20.4111	2.4111	4.1000
0.8062	18.0000	20.4111	2.4111	4.1000
0.8062	17.0000	20.4111	3.4111	4.1000
0.8062	12.0000	20.4111	8.4111	4.1000
0.9685	24.0000	16.6836	-7.3164	3.8000
0.9685	23.0000	16.6836	-6.3164	3.8000
0.9685	21.0000	16.6836	-4.3164	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

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

Error Summary

No Errors

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		

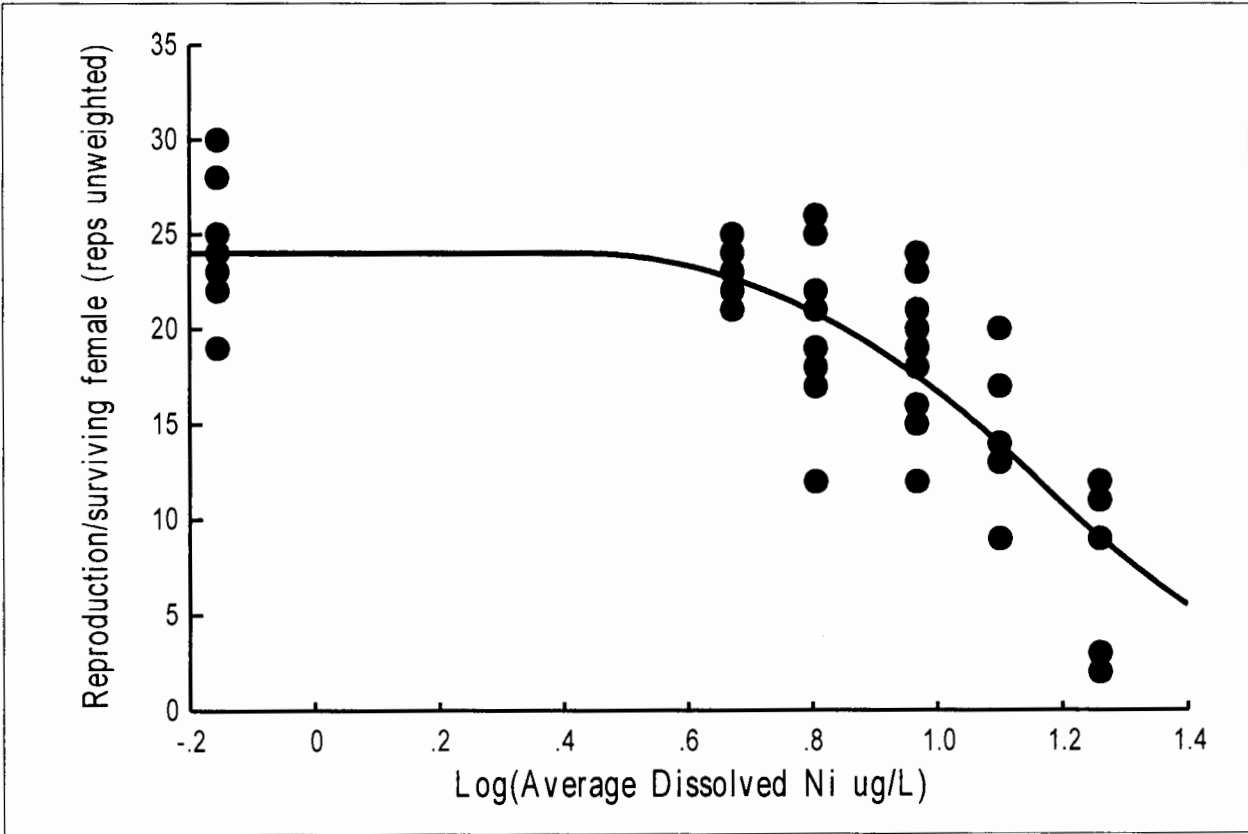
Data Summary

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
LogX 50	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

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.

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

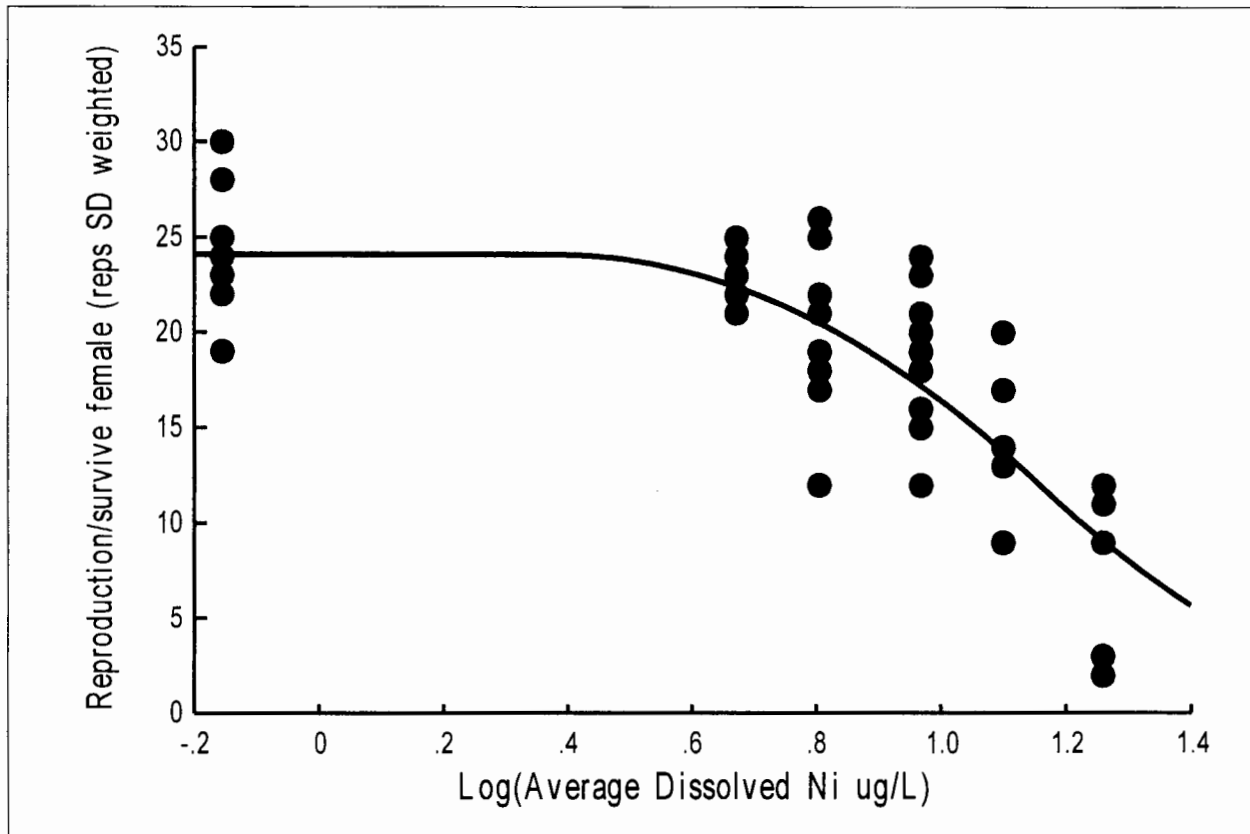
Data Summary

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

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
0.6721	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

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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Nominal Treatment µg/L Ni	Measured (Average) Dissolved Ni µg/L	Rep	Survival	total # of neonates	Per original female		Per surviving female	
					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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

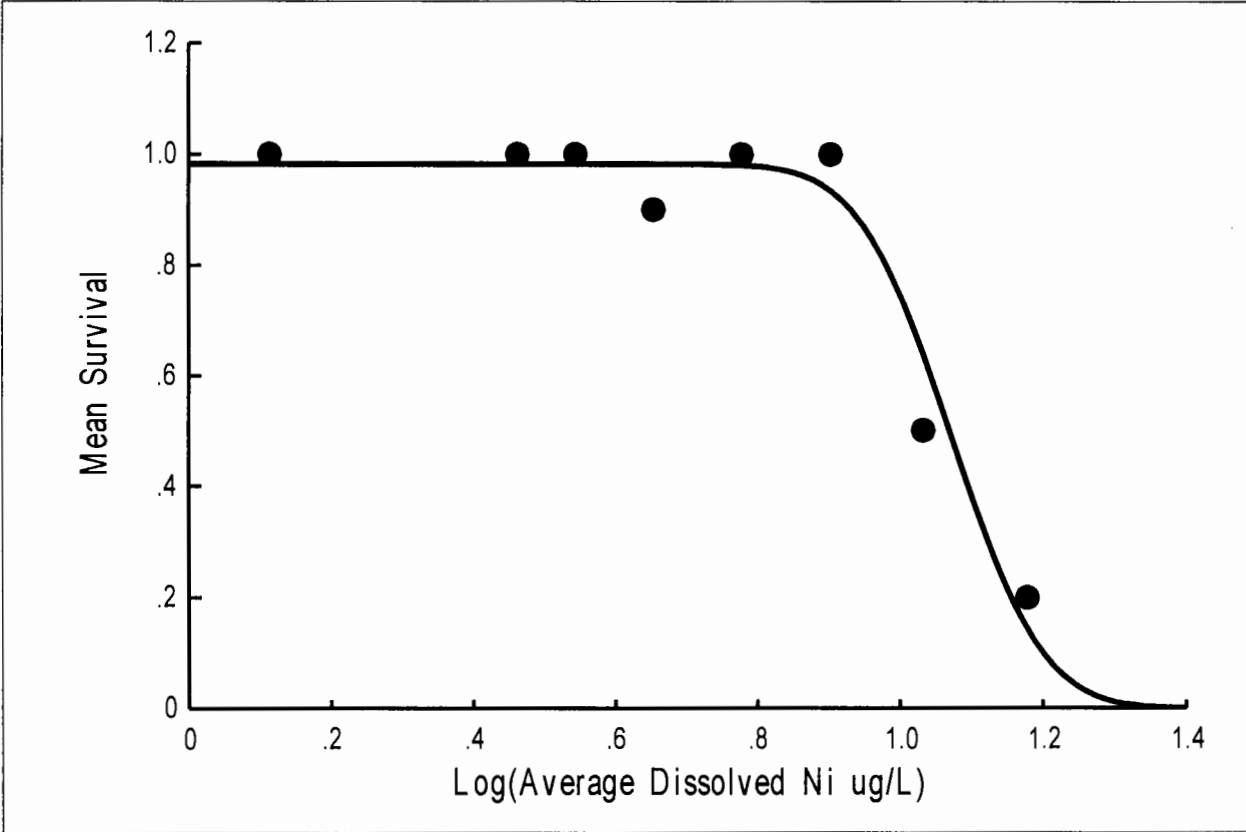
Nickel WER without DOC

Test ID: Ni WER 1132R CDC

Nominal Treatment µg/L Ni	Measured (Average) Dissolved Ni µg/L	Rep	Survival	total # of neonates	Per original female		Per surviving female	
					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
LogX 50	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

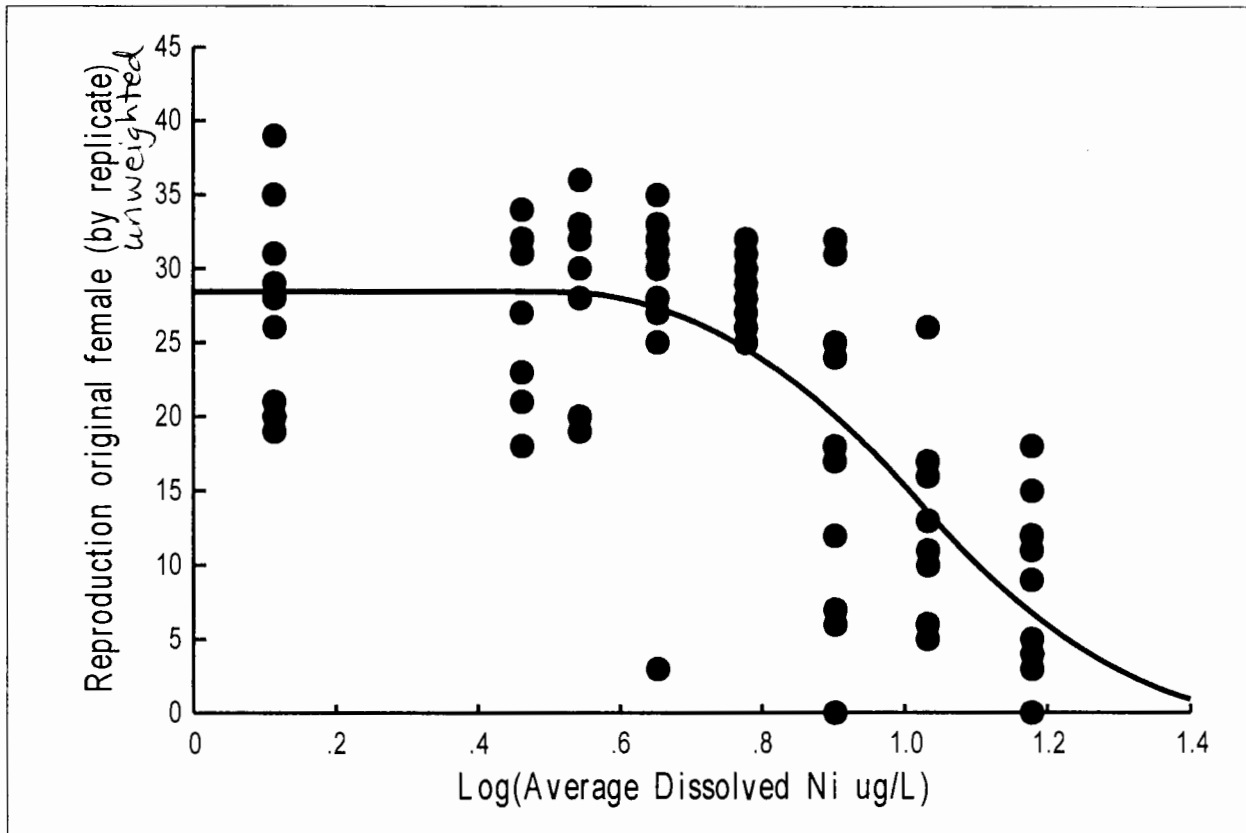
Data Summary

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

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

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

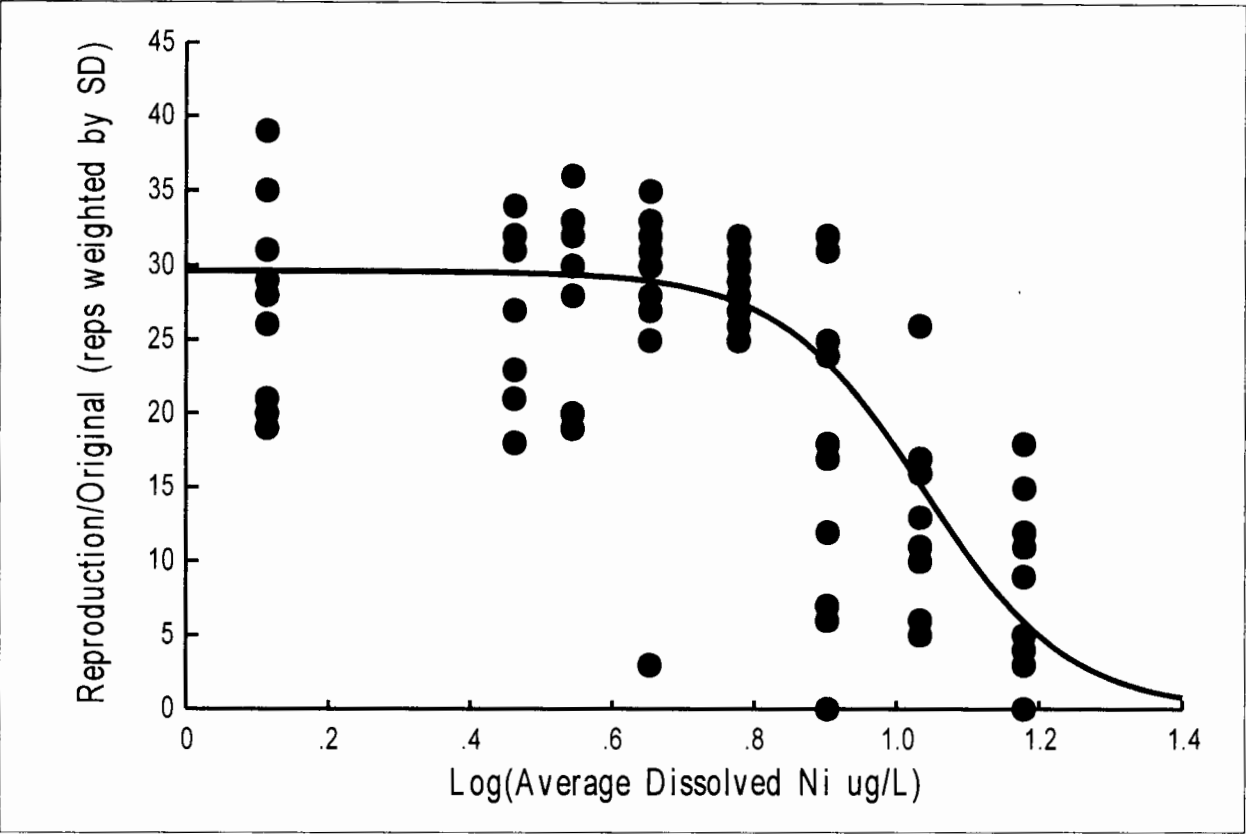
Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

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
LogX 50	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

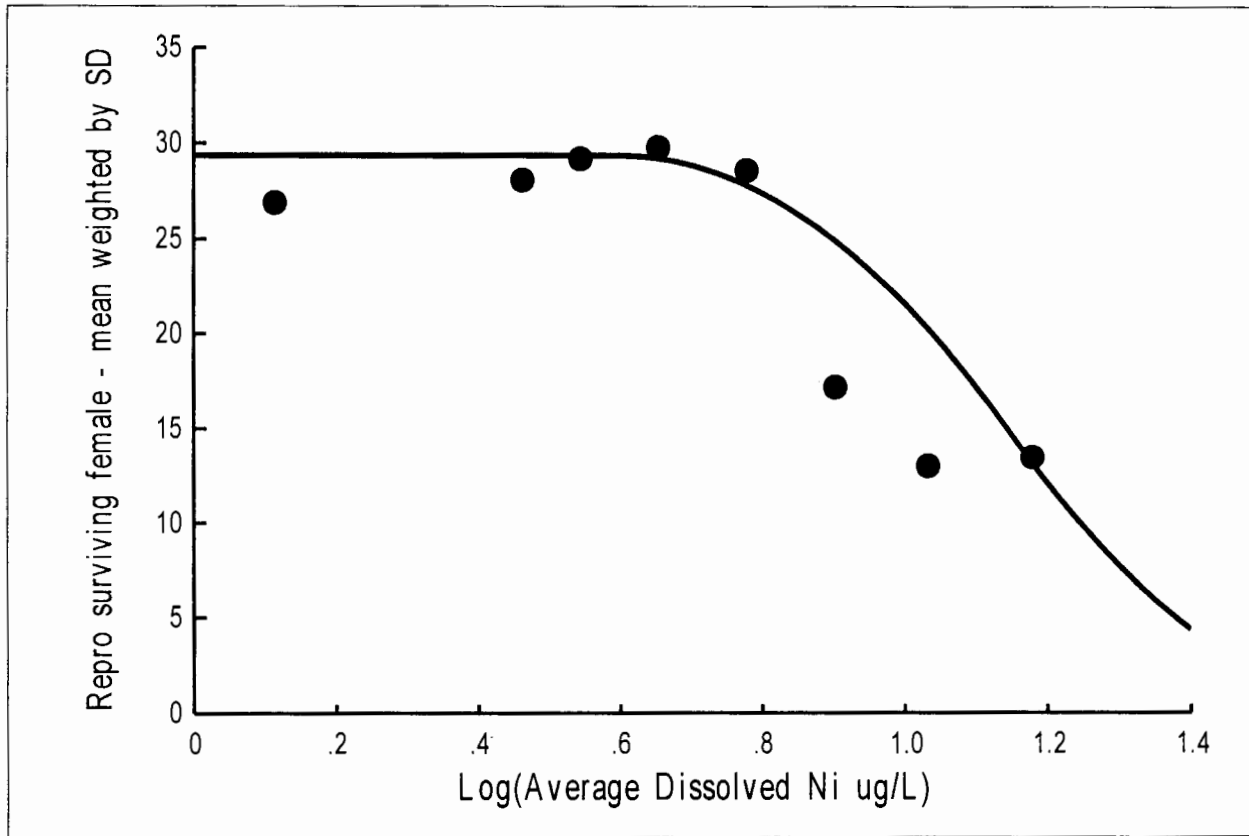
Chronic toxicity of a Ni-spiked simulated effluent - no DOC added: Ni WER 1132R

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

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		

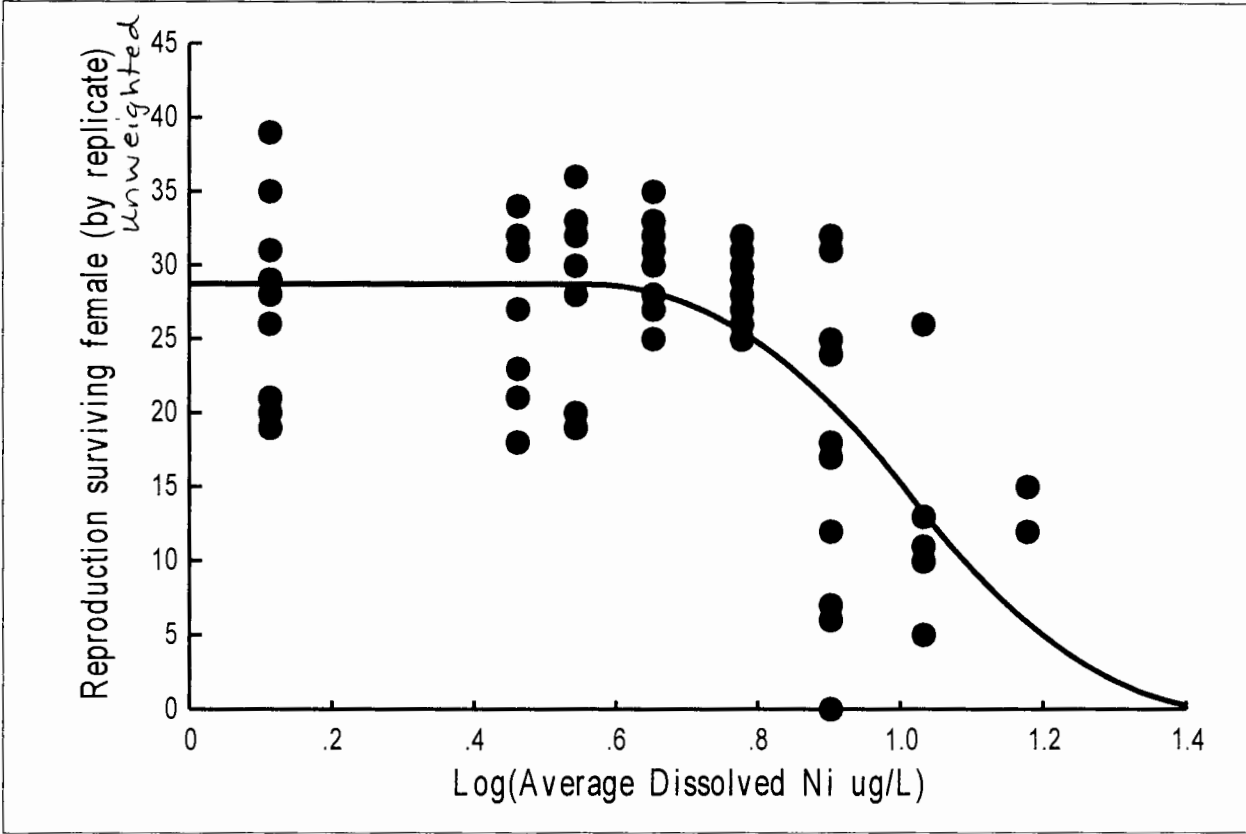
Data Summary

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

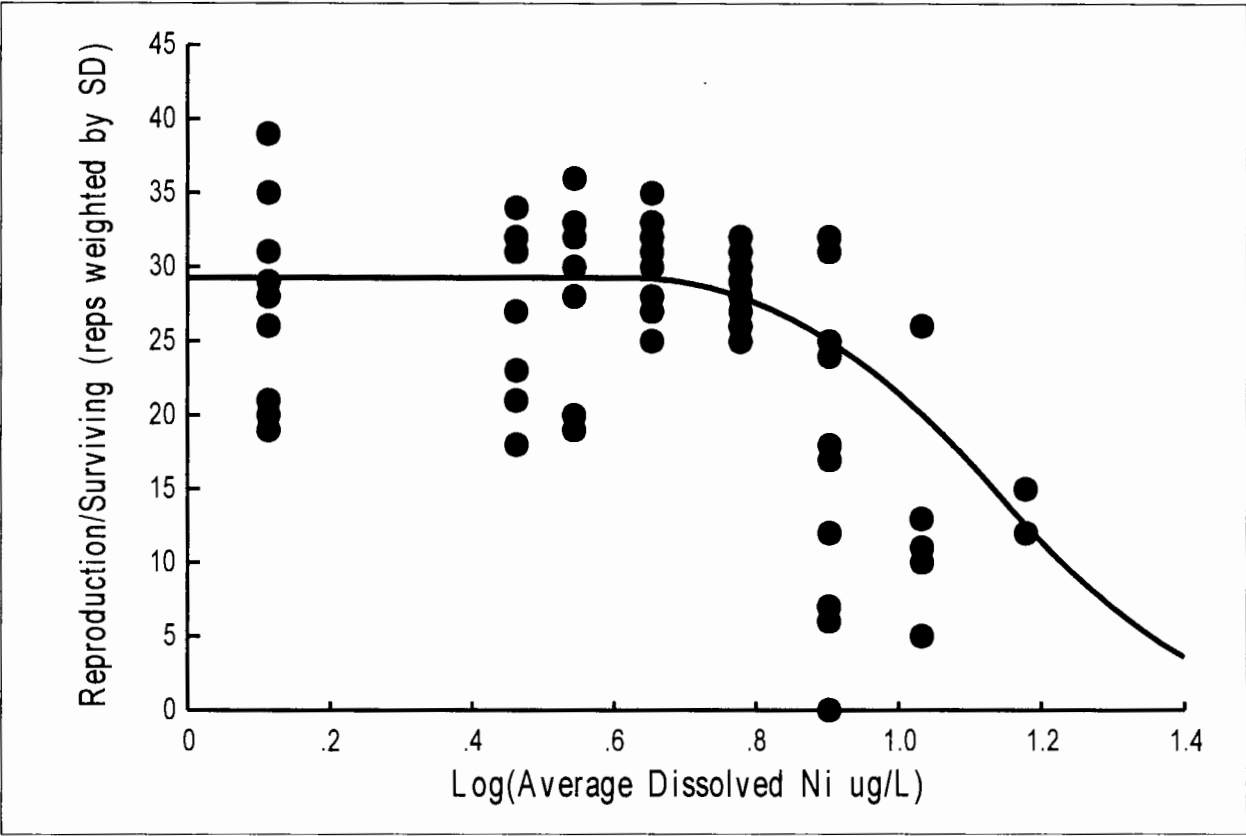
Data Summary

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

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
LogX 50	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

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

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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

Nominal Treatment µg/L Ni	Measured (Average) Dissolved Ni µg/L	Rep	Survival	total # of neonates	Per original female		Per surviving female	
					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

Electronic Filing: Received, Clerk's Office 4/20/2018

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

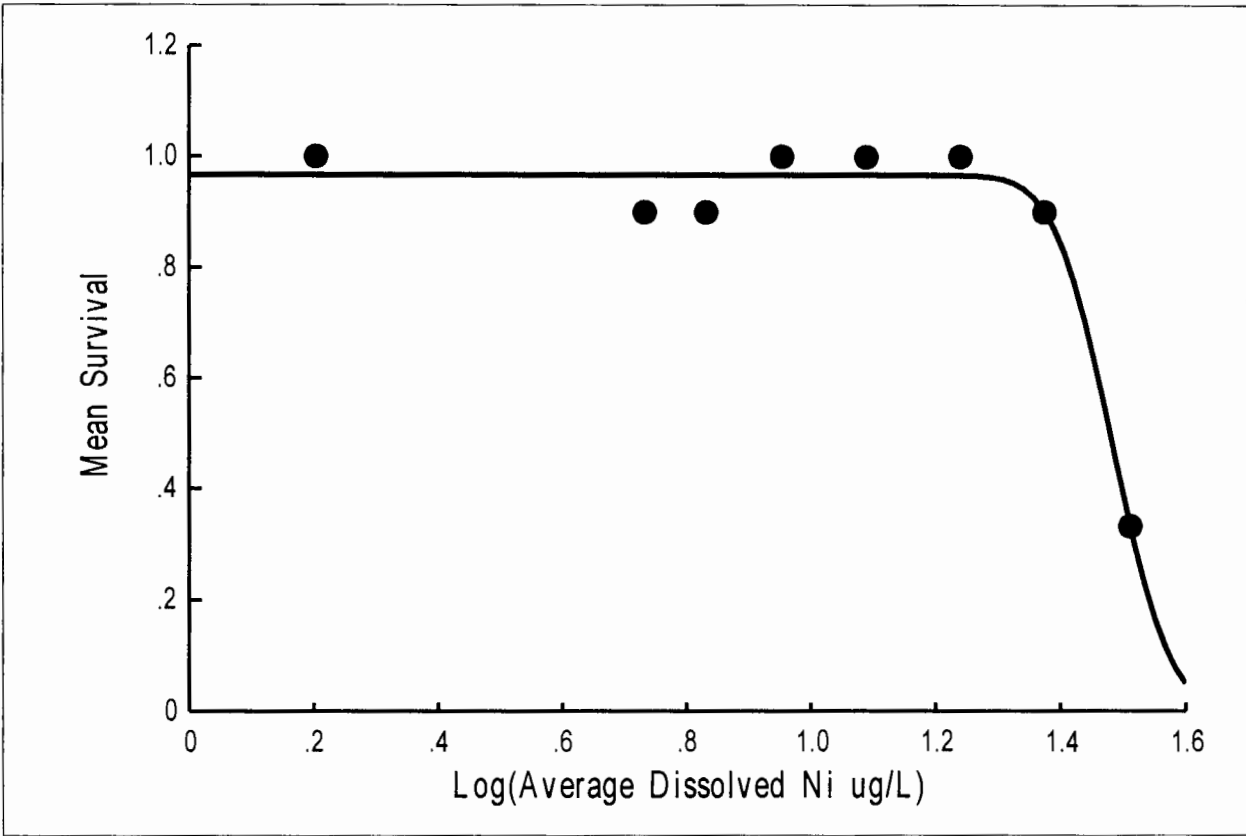
Nickel WER with added DOC

Test ID: Ni WER 1126 CDC

Nominal Treatment µg/L Ni	Measured (Average) Dissolved Ni µg/L	Rep	Survival	total # of neonates	Per original female		Per surviving female	
					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
LogX 50	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

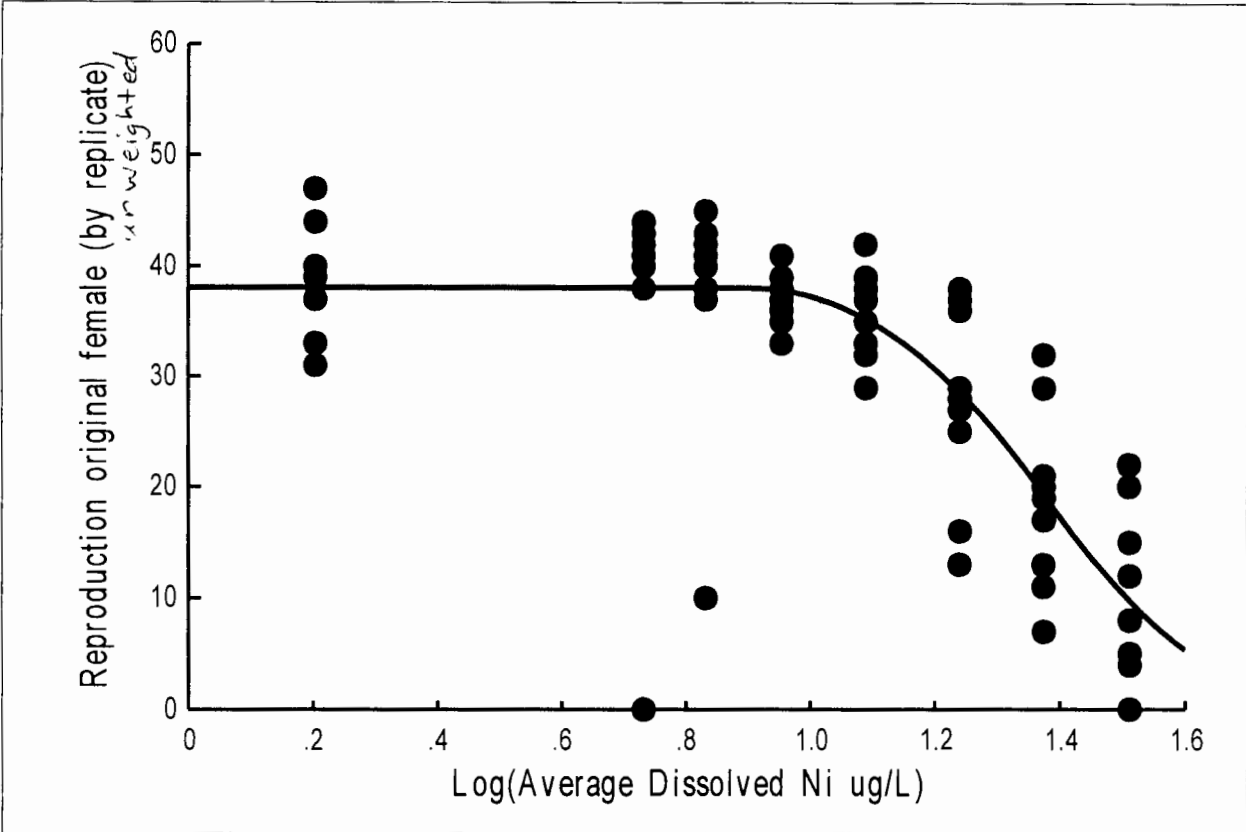
Data Summary

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

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
LogX 50	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.

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.

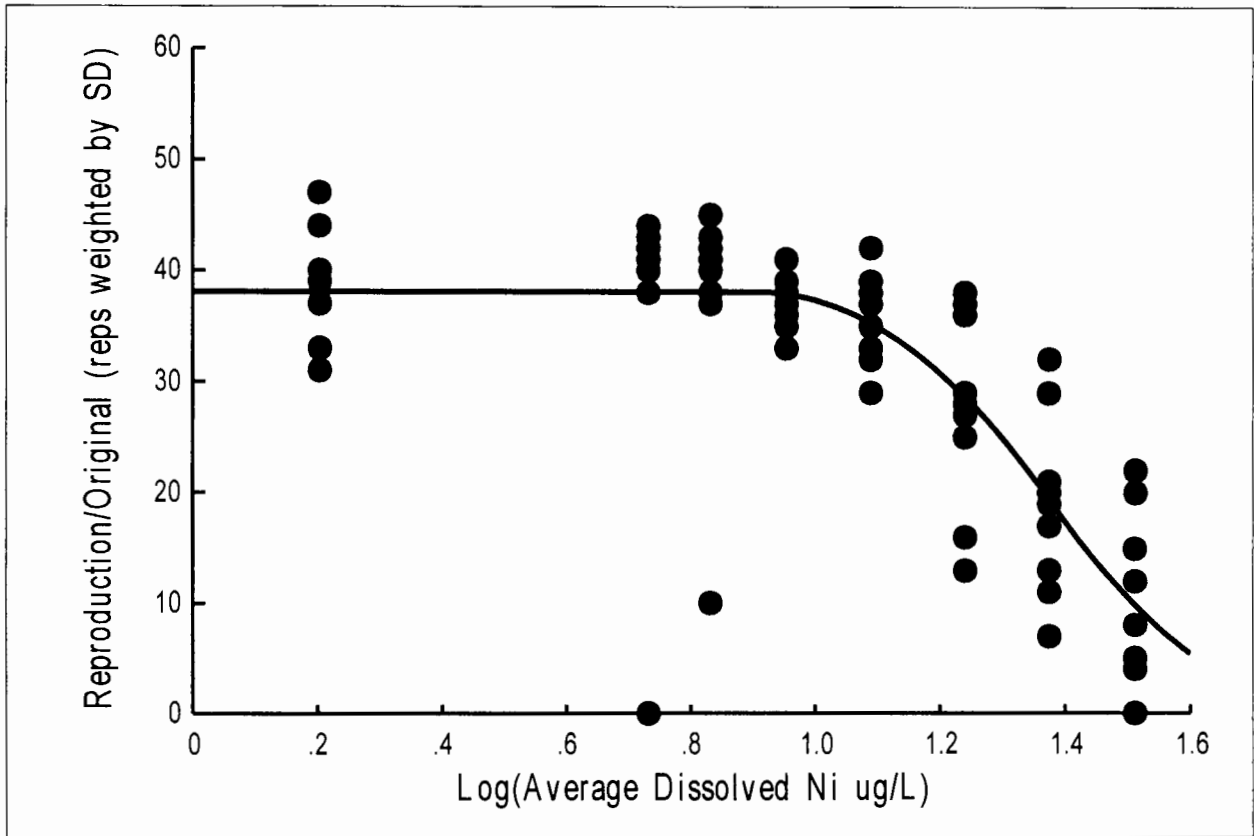
ASC 8/1/17

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

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
LogX 50	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

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

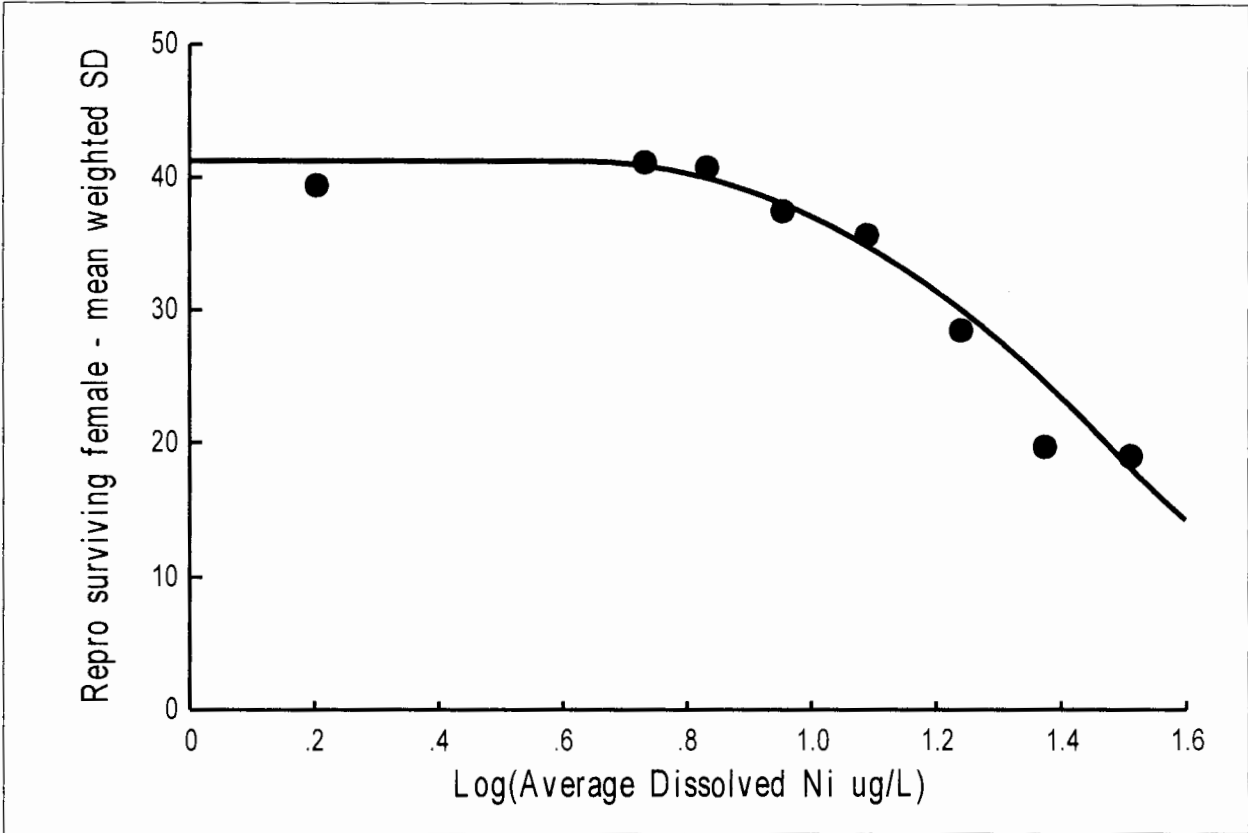
Chronic toxicity of a Ni-spiked simulated effluent with DOC: Ni WER 1126 CDC

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

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		

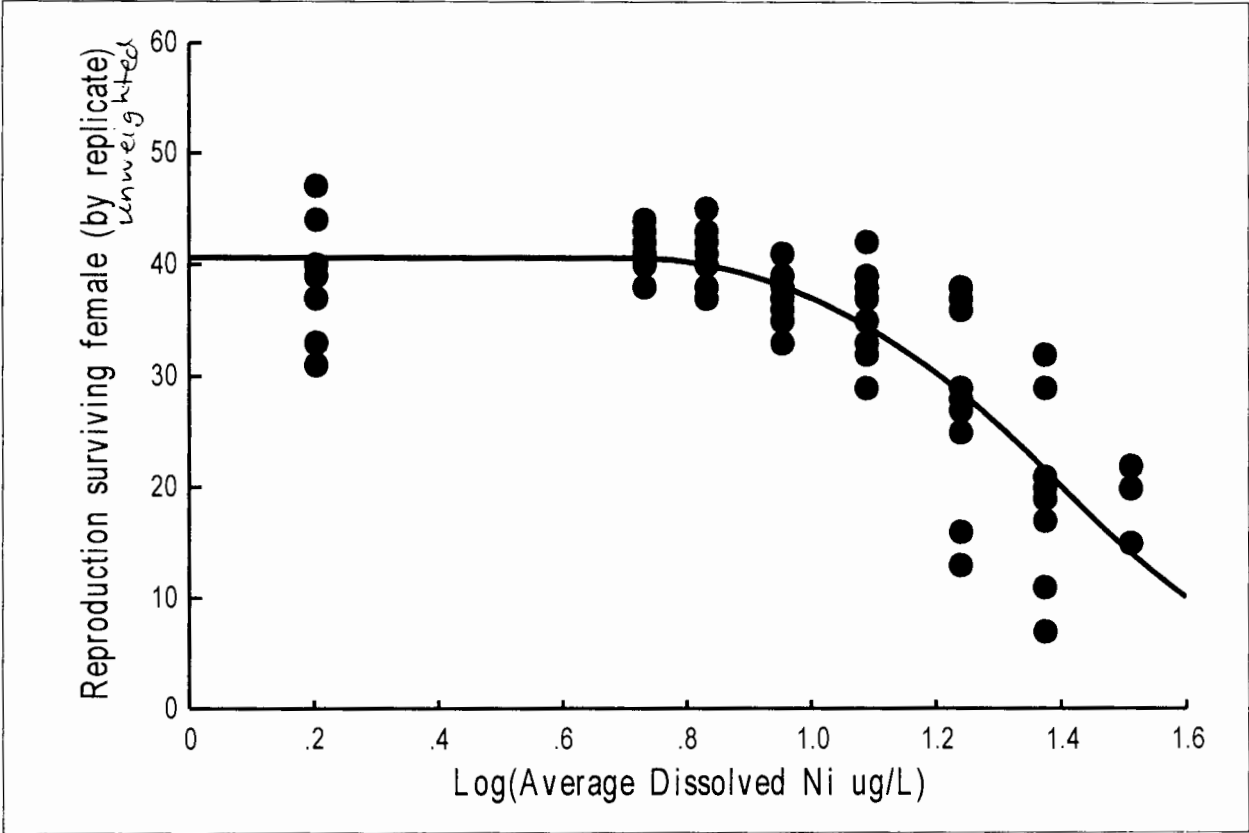
Data Summary

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

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
LogX 50	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

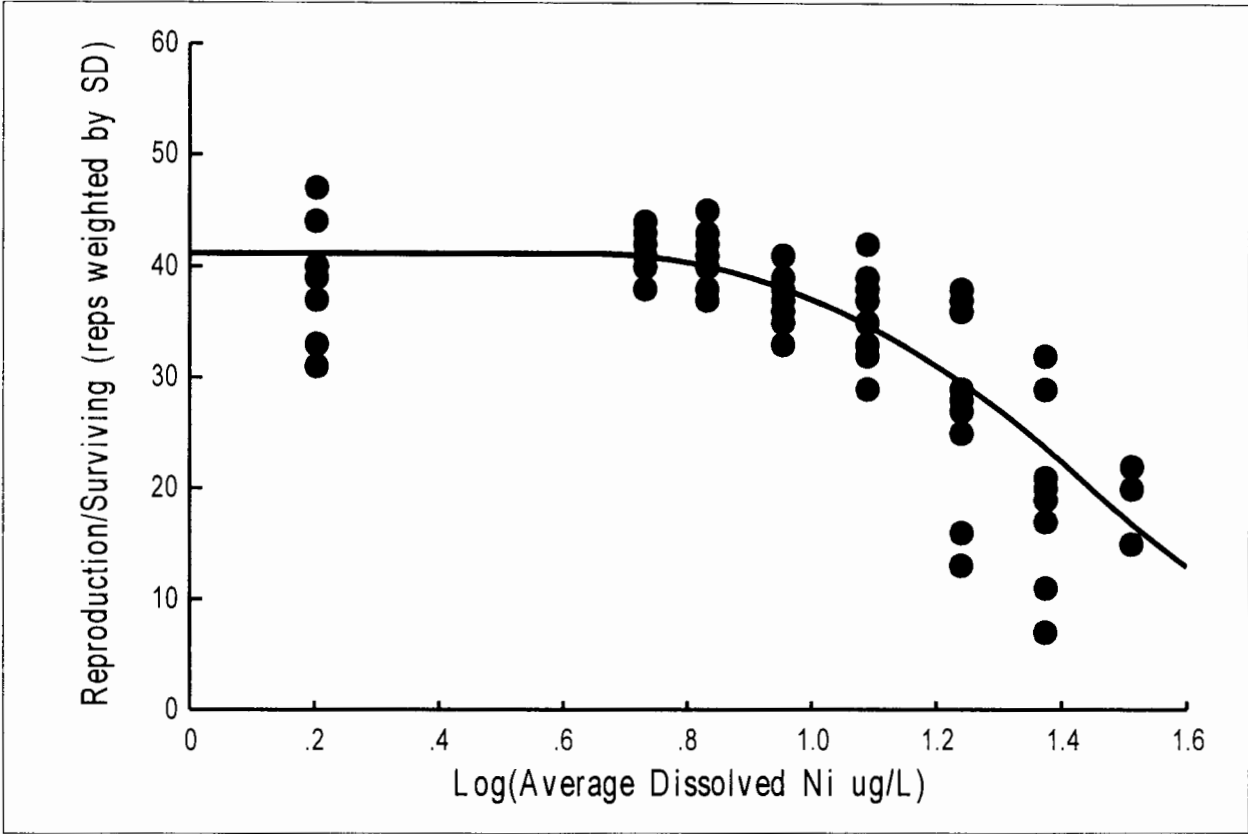
Data Summary

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

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

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

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; data from Hoang et al. 2004 for less than 1 day old *Pimephales promelas* at a pH of 9; 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 A2-2. DOC and Ni effect concentrations used in the ANCOVA analysis.

Group	Measured Ni EC20 (mg/L)	DOC (mg/L)
Hoang et al. 2004 <i>P. promelas</i> (<1d) pH9)	2.28	2.02
Hoang et al. 2004 <i>P. promelas</i> (<1d) pH9)	2.92	6.24
Hoang et al. 2004 <i>P. promelas</i> (<1d) pH9)	3.50	8.63
Kozlova et al. 2009 <i>D. pulex</i> (NRNOM)	1.47	1.53
Kozlova et al. 2009 <i>D. pulex</i> (NRNOM)	1.64	2.84
Kozlova et al. 2009 <i>D. pulex</i> (NRNOM)	3.05	9.80
Kozlova et al. 2009 <i>D. pulex</i> (NRNOM)	5.22	16.50
Kozlova et al. 2009 <i>D. pulex</i> (SRNOM)	1.00	0.50
Kozlova et al. 2009 <i>D. pulex</i> (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 A2-3. Results of the ANCOVA analysis.

	df	Sum Squares	Mean Square	F value	Pr(>F)
log ₁₀ (DOC)	1	1.3622	1.36216	308.5671	6.168e-05
Group	3	8.0828	2.69426	610.3242	8.903e-06
log ₁₀ (DOC) * Group	3	0.0371	0.01236	2.8008	0.1726
Residuals	4	0.0177	0.00441		

Because the significance of the interaction term (log₁₀(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:

$$\log_{10} Ni = 0.3260 * \log_{10} DOC + Group \text{ intercept}$$

Table A2-4. Summary of statistical results from the ANCOVA analysis.

	Estimate	Standard Error	t value	Pr(> t)
log ₁₀ (DOC) slope	0.32605	0.04899	5.537	0.000289
Hoang et al. 2004 P. promelas (<1d pH9) intercept	0.23485	0.06094	3.854	0.006260
Kozlova et al. 2009 D. pulex (NRNOM) intercept	0.16417	0.05631	2.915	0.022489
Kozlova et al. 2009 D. pulex (SRNOM) intercept	0.08858	0.06057	1.462	0.187034
OSU C. dubia reproduction intercept	-2.07852	0.06567	-31.649	8.12e-09

The model and data are shown in Figure A2-1. The model is significant ($p = 1.12e-07$) with a multiple R^2 of 0.9942.

Transcript from R:

```
> DOC.sub5[, c("group", "meas_Ni_mg.L", "DOC_mg.L")]
      group meas_Ni_mg.L DOC_mg.L
1 Hoang et al. 2004 P. promelas (<1d) pH9) 2.283000 2.02
2 Hoang et al. 2004 P. promelas (<1d) pH9) 2.924000 6.24
3 Hoang et al. 2004 P. promelas (<1d) pH9) 3.500000 8.63
4 Kozlova et al. 2009 D. pul ex (NRNOM) 1.467325 1.53
5 Kozlova et al. 2009 D. pul ex (NRNOM) 1.643404 2.84
6 Kozlova et al. 2009 D. pul ex (NRNOM) 3.052036 9.80
7 Kozlova et al. 2009 D. pul ex (NRNOM) 5.223677 16.50
8 Kozlova et al. 2009 D. pul ex (SRNOM) 0.997781 0.50
9 Kozlova et al. 2009 D. pul ex (SRNOM) 2.934650 10.00
10 Kozlova et al. 2009 D. pul ex (SRNOM) 2.817264 19.80
11 OSU C. dubia reproducti on 0.008000 0.54
12 OSU C. dubia reproducti on 0.016100 12.20
>
>
> l.m.cov = lm(log10(meas_Ni_mg.L) ~ log10(DOC_mg.L) + group + log10(DOC_mg.L):group,
+ data = DOC.sub5)
> anova(l.m.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  1.3622  1.36216  308.5671 6.168e-05 ***
group           3  8.0828  2.69426  610.3242 8.903e-06 ***
log10(DOC_mg.L):group  3  0.0371  0.01236   2.8008  0.1726
Residuals      4  0.0177  0.00441
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
>
>
> l.m.sub = lm(log10(meas_Ni_mg.L) ~ log10(DOC_mg.L) + group + 0,
+ data = DOC.sub5)
> summary(l.m.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:
    Min       1Q   Median       3Q      Max
-0.096227 -0.058777  0.000636  0.031307  0.156851
```

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
log10(DOC_mg. L)	0.32605	0.04899	6.655	0.000289	***
groupHoang et al. 2004 P. promelas ((<1d) pH9)	0.23485	0.06094	3.854	0.006260	**
groupKozlova et al. 2009 D. pulex (NRNOM)	0.16417	0.05631	2.915	0.022489	*
groupKozlova et al. 2009 D. pulex (SRNOM)	0.08858	0.06057	1.462	0.187034	
groupOSU C. dubia reproduction	-2.07852	0.06567	-31.649	8.12e-09	***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.08844 on 7 degrees of freedom

Multiple R-squared: 0.9942, Adjusted R-squared: 0.9901

F-statistic: 241.5 on 5 and 7 DF, p-value: 1.12e-07

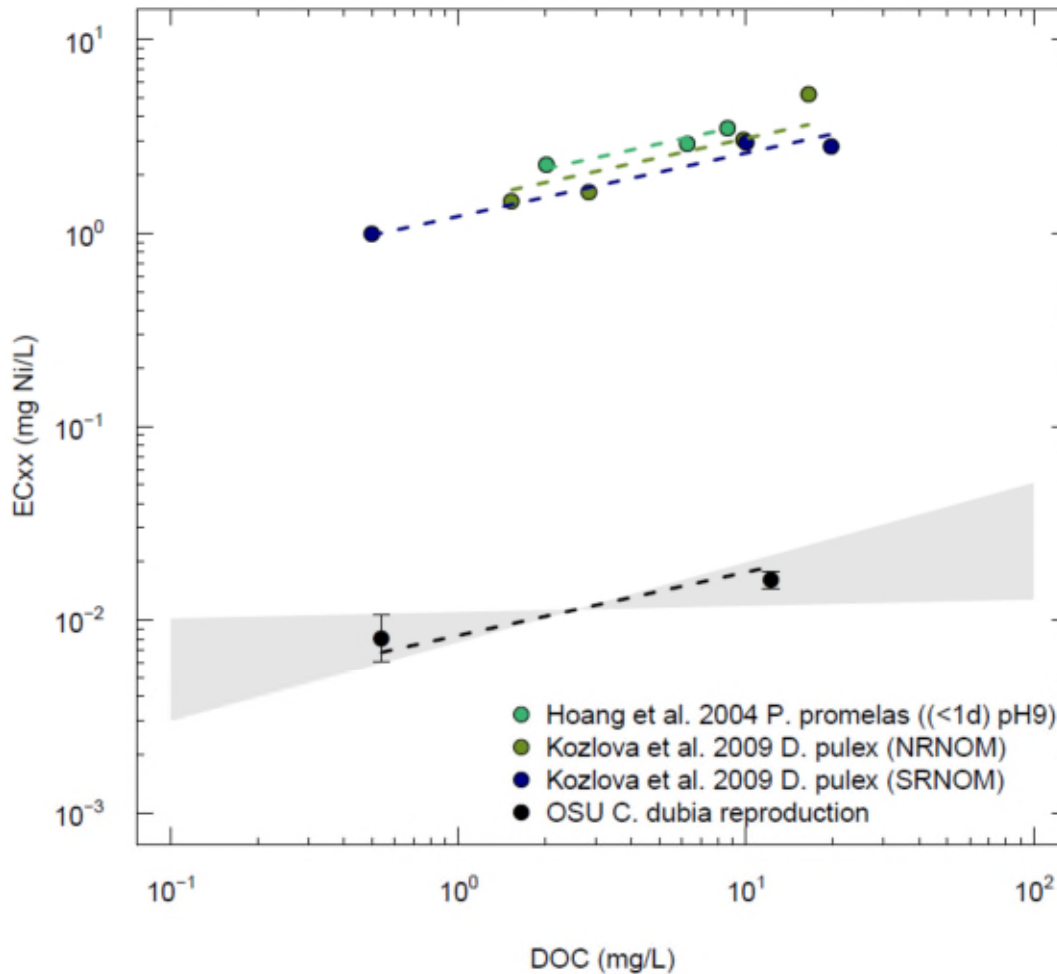


Figure A2-1. The overall best DOC regression as determined by the ANCOVA analysis is shown (dashed line) compared to the data used to develop the overall relationship (filled circles). The range of feasible slopes for the OSU data, considering the confidence limits on the individual points, is shown as a shaded region.

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