Veedersburg WWTP WET Testing Study Plan

Project Description: Chronic WET testing of Veedersburg WWTP final effluent using three different dilution waters to determine their mitigating effect on the toxicity of nickel to *C. dubia*.

Participating WET testing laboratories: Great Lakes Environmental Center (Columbus, OH), Pace Analytical Madisonville, KY and Biomonitor, Indianapolis, IN

Veedersburg WWTP effluent composite sample dates: Due to present circumstances with COVID19, the start of sampling has been postponed until a later date. We will update you once that date is scheduled. Sampling will begin on a Sunday-Monday(I), Tuesday-Wednesday(II) and Thursday-Friday(III). The date of first use of each effluent sample for testing by the participating laboratories will occur on Tuesday(I), Thursday(II) and Saturday(III). First use of each effluent sample will occur prior to the expiration of the 36-hour WET testing sample hold time.

Veedersburg WWTP effluent composite other testing: The effluent will also be collected and sent to 3 laboratories for metals and other non-WET test analytes. Samples will be sent to Pace, Element Labs, and Astbury and analyzed for the following using EPA Method 200.8: Nickel, cadmium, chromium, copper, lead, and zinc. The effluent samples will also be analyzed for chloride, sulfate, and calcium and magnesium hardness. Dissolved organic carbon in the effluent will be analyzed by Biomonitor and Element Labs.

Coal Creek upstream receiving water sample collection: A one-time grab sample of the receiving water will be collected upstream and outside of the influence of the Veedersburg WWTP effluent mixing zone. The receiving stream sample should be collected immediately prior to the test, but never more than 96 hours before the test begins. Each of the participating laboratories should receive at least seven liters of the receiving water sample, which will be delivered along with the first composite effluent sample, and will be used for the duration of the chronic toxicity tests where receiving water is the dilution water. Note: (~6 gallons to be collected, total)

Coal Creek samples will be submitted to Element Labs and Biomonitor to be analyzed for dissolved organic carbon. Coal Creek samples will also be submitted to Element Labs and Astbury to be analyzed for the following using EPA Method 200.8: Nickel, cadmium, chromium, copper, lead, and zinc. In addition, to be analyzed for chloride, sulfate, and calcium and magnesium hardness.

Sample receipt, handling and storage by the participating laboratories: The three effluent samples and the one-time receiving water grab sample will be logged in and assigned a laboratory identification number. At the time of receipt, temperature, pH, dissolved oxygen and conductivity will be measured (measurements will be performed strictly for bioassay testing and not for reportable NPDES compliance data purposes). After the effluent composite sample is warmed to test temperature, pH, dissolved oxygen, conductivity, alkalinity, dissolved organic carbon, sulfide, chloride, and calcium and magnesium hardness will be measured. Toxicity testing will be initiated within 36 hours of sample collection. The Coal Creek receiving water sample will be filtered through a Nitex screen (37 - 60 μm) prior to testing to remove planktonic crustaceans or predators while the Veedersburg effluent samples will not be filtered through a screen). Samples water not used during testing will be stored at 0 to 4°C.

Three water types are to be use as the dilution and primary control waters for the chronic WET tests: 1. Moderately hard synthetic laboratory water (Target hardness range: 80-100 mg/L), 2. Very hard synthetic laboratory water with a modified Ca/Mg ratio (Target hardness range: 280-320 mg/L) and 3. Coal Creek upstream receiving water sample, as previously explained.

1. Moderately hard synthetic laboratory water will be prepared in accordance with guidance provided by the US EPA Chronic Testing Method (EPA-821-R-02-013). See the table below for guidance in preparation of the moderately hard synthetic laboratory water. Prepared water should be allowed to mix and aerate for 24-hours prior to use.

Specifications to formulate one liter of moderately hard synthetic laboratory water (mg/L)

$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl	Na_2SeO_4	Target Hardness	Target Alkalinity
96.0 mg	60.0 mg	60.0 mg	4.0 mg	2.0 mg	80-100 mg/$CaCO_3$/L	57-64 mg/$CaCO_3$/L

2. Very hard synthetic laboratory water will be prepared in accordance with guidance provided by the US EPA Chronic Testing Method (EPA-821-R-02-013). However, the ratio of the calcium to magnesium will mimic the measured calcium and magnesium concentration of Coal Creek. Recent samples collected from Coal Creek had measured calcium hardness concentrations of 179, 176, 177 and 174 mg/L and measured magnesium hardness concentrations of 89.1, 87.5, 88.0 and 90.4 mg/L. From the average measured concentrations, the calculated ratio for calcium and magnesium is approximately 2.7:1. As a result, the US EPA guidance for formulating very hard synthetic laboratory water has been modified to incorporate a 2.7:1 calcium to magnesium ratio. Prepared water should be allowed to mix and aerate for 24-hours prior to use.

Specifications to formulate one liter of very hard synthetic laboratory water with a 2.7:1 Ca:Mg ratio (mg/L)

$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl	Na_2SeO_4	Target Hardness	Target Alkalinity
384 mg	323 mg	136 mg	16.0 mg	2.0 mg	280-320 mg/$CaCO_3$/L	225-245 mg/$CaCO_3$/L

The chronic WET test specifications: Testing specifications for the chronic WET testing can be found in the table below. Please note that the test concentrations (bolded) are based on the recommendation by John Elliot of IDEM and are intended to more accurately determine toxicity above the 33% effluent test concentration that may be caused by the presence of nickel in the Veedersburg final effluent.

1.	Test Species and Age:	*Ceriodaphnia dubia*; <24 h (all individuals are within <8 h)
2.	Test Type and Duration:	Static Renewal; 6-7 days (3 brood test)
3.	Test Temperature (°C):	25 ± 1
4.	Light Quality:	Ambient Laboratory; 10-20 µE/m2/s
5.	Photoperiod:	16 h Light, 8 h darkness
6.	Feeding Regime:	*Raphidocelis subcapitata* and YTC
7.	Size of Test Vessel:	30 mL
8.	Volume and Depth of Test Solutions:	15 mL; 20 mm
9.	No. of Test Organisms per Test Vessel:	1
10.	No. of Test Vessels per Test Solution:	10
11.	Total No. of Test Organisms per Test Solution:	10
12.	Test Concentrations (as percent by volume effluent):	**100, 80, 50, 33.3,12.5, 6.25**
13.	Renewal of Test Solutions:	Daily
14.	Dilution and Primary Control Waters:	Moderately hard synthetic laboratory water, Very hard synthetic laboratory water with a modified Ca/Mg ratio and Coal Creek upstream receiving water
15.	Secondary Control Water:	Diluted Mineral Water (DMW), refer to EPA Method 821-R-02-013 Section 7.1.1.2, 7.1.3 and 7.2.
16.	Aeration:	None
17.	Endpoints Measured:	Survival and Reproduction
18.	Test Acceptability Criteria:	80% or greater survival of all control organisms and an average of 15 or more young per surviving female in the controls. 60% of the surviving control females must produce three broods.