UPPER COLUMBIA RIVER

Acute Water Exposures of Cadmium, Copper, and Zinc to Early Life-Stages of White Sturgeon (*Acipenser transmontanus*)

Prepared for

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1 PURPOSE AND OBJECTIVES

The purpose of this study was to evaluate the acute toxicity to early life-stages (ELS) of white sturgeon (*Acipenser transmontanus*) of water-borne cadmium, copper, and zinc. Specifically, this study was designed to provide data needed to answer the following questions:

1. What concentrations of cadmium, copper, and zinc are acutely toxic to ELS of white sturgeon as determined using standard 96-hour toxicity tests?
2. Are ambient water quality criteria (AWQC) for cadmium, copper, and zinc protective of ELS of white sturgeon?

2 METHODS

2.1 TEST SPECIES AND SOURCE

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery (KTH) located in Fort Steele, British Columbia on July 15, 2008. Hatchery staff collected eggs from two female and six male breeding individuals captured in the Columbia River (CR) near Waneta, Canada (Ron Ek 2008, pers. comm.). Fertilization of the eggs was harmonized in the hatchery by injecting the sturgeon with a gonadotropin analog on two subsequent days. Embryos were transported in oxygenated bags and received at the testing laboratory within 4 to 8 hours of fertilization. Embryos were acclimated to test waters for 1-hour, before being incubated in McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL). Hatching began and ended on July 21 and 25, respectively; approximately 6 to 10 days post-fertilization.

2.2 TEST MATERIALS

Stock solutions were prepared using copper (II) sulfate pentahydrate (Chemical Abstracts Service [CAS] number 7758-99-8; purity 99.995 percent), cadmium chloride hemi-pentahydrate (CAS number 7790-78-5; purity 99.999 percent), and zinc chloride (CAS number 7646-85-7; purity 98 percent). Chemicals were obtained from Sigma-Aldrich (Oakville, ON, Canada). All chemicals were directly dissolved in test waters and allowed to equilibrate for approximately 72 hours prior to testing.

Nominal concentrations in micrograms per liter (μg/L) to be tested were:

Cadmium = 0, 1.95, 7.81, 31.25, 125, and 500 μg/L

Copper = 0, 1.56, 6.25, 25, 100, and 400 μg/L

Zinc = 0, 19.5, 78.1, 312.5, 1,250, and 5,000 μg/L

2.3 EXPOSURE METHODS

Acute 96-hour toxicity tests were conducted at the Aquatic Toxicology Research Facility, University of Saskatchewan (U of S), Saskatoon, Saskatchewan; and adjacent to the Columbia River (CR) at a location upstream of the Teck Metals Ltd. Trail smelter facility (the facility) along the east bank of the CR (49°07′01.32″ N; 117°43′27.25″ W) at river mile (RM) 758.

All exposures were initiated at 8 to 10 days post hatch (dph) and were conducted under static renewal conditions in decontaminated 0.5 liter (L) polypropylene containers per American Society for Testing and Materials (ASTM) guidelines for testing ELS of fish (ASTM 2009 – E1241-05 and ASTM 2007 – E729-96). As such, 50 percent of the test solution was replaced every 12 hours. All exposures were conducted under an illumination cycle of 16 light to 8 dark hours; and a target temperature of 16 ± 1 degrees Celsius (°C).

Mortality and routine observations (e.g., behavior) were recorded at time (t) = 0, t = 24 hours, t = 48 hours, t = 72 hours, and t = 96 hours. At the conclusion of the test, all sturgeon larvae were euthanized using tricaine methanesulfonate (MS222), measured, weighed, and fixed in 10 percent buffered formalin.

2.3.1 U of S Toxicity Testing

Test solutions were prepared in reverse osmosis (RO) water adjusted to a water hardness of approximately 70 milligrams as calcium carbonate per liter (mg/L $CaCO_3$) by adding laboratory (i.e., dechlorinated City of Saskatoon water) in a 1:1 ratio. Water quality parameters measured at the beginning and end of the study included temperature, dissolved oxygen (DO), pH, conductivity, hardness, ammonia, nitrate, nitrite, sulfate, phosphate, total chlorine, dissolved organic carbon (DOC) and dissolved metal (cadmium, copper, and zinc) concentrations.

2.3.2 Columbia River Toxicity Testing

Test solutions were prepared by mixing stock solutions directly with CR water and allowed to equilibrate for a minimum of 72 hours. Water quality parameters measured at the beginning and end of the study included hardness, ammonia, nitrate, nitrite, sulfate, and phosphate. Temperature, DO, pH, and conductivity were measured daily. Dissolved metal concentrations were measured at the beginning of the experiment in the stock solutions only.

2.4 WATER CHEMISTRY AND WATER QUALITY

Routine water quality parameters (i.e., temperature, pH, DO, and conductivity) were recorded daily with symphony electrodes (VWR, Cat # 11388-328). Hardness, alkalinity, ammonia, nitrate, nitrite, chlorine, sulfate, sulfide, and phosphate were recorded at the initiation and termination of the study using LaMotte colorimetric and titrator test kits. In addition, for tests conducted at the U of S laboratory, composite water samples were collected from all replicate exposure chambers of a treatment group at the beginning and end of the test, and analyzed for dissolved metals (cadmium, copper, and zinc). Samples were collected using acid-cleaned polyethylene bottles, filtered through a 0.45 micrometer (μm) polycarbonate filter with Nalgene® filter holders and receivers; acidified with ultrapure nitric acid to a pH <2 standard units (s.u.), and maintained at approximately 4°C for shipment to the analytical laboratory (Liber Laboratory, Toxicology Centre; U of S).

Dissolved metal analyses were performed using inductively coupled plasma mass spectrometry (ICP-MS) following U.S. Environmental Protection Agency (EPA) Method ILM05.2D (Creed et al. 1994). DOC analysis was performed using a total organic carbon analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, Ontario, Canada). A summary of the samples collected, blanks, analytical methods and associated method detection limits is provided (Tables 1 and 2).

2.5 DATA ANALYSIS AND STATISTICS

Data are summarized as the mean ±1 standard deviation (SD). Fish mortality was analyzed by comparing the proportion of dead fish in each of the three exposure chambers of a given metal concentration to that of the controls. Data were tested for normality using the Shapiro-Wilk test. All data were tested for normality or approximated normal distribution and analysis of variance (ANOVA) and Bonferroni *post hoc* tests were used to detect significant differences between treatment and control groups. Concentrations at which 50 percent mortality occurred (LC50) were calculated either using a PROBIT model or a log-linear regression model, as appropriate. Statistical significance was accepted when $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 EXPOSURE VERIFICATION

A summary of nominal and measured metal concentrations is presented in Table 3. As indicated by the data, average measured concentrations were generally in good agreement with target concentrations.

Table 1. Samples Collected, Analytical Methods and Associated Method Detection Limits

Treatment Groups	Parameter	Sample Type	Dates	Method	Unit	LOD
Laboratory						
Y, B1-5, O1-5, P1-5	Cadmium	Composite	7/31/2008; 8/4/2008	EPA ILM05.2D	mg/L	0.0031
Y, B1-5, O1-5, P1-5	Copper	Composite	7/31/2008; 8/4/2008	EPA ILM05.2D	mg/L	0.068
Y, B1-5, O1-5, P1-5	Zinc	Composite	7/31/2008; 8/4/2008	EPA ILM05.2D	mg/L	0.18
Y, B1-5, O1-5, P1-5	Temperature	Individual	7/31/2008; 8/4/2008	VWR Symphony 14002-860	˚C	0
Y, B1-5, O1-5, P1-5	pH	Individual	7/31/2008; 8/4/2008	VWR Symphony 14002-860	s.u.	0
Y, B1-5, O1-5, P1-5	DO	Individual	7/31/2008; 8/4/2008	VWR Symphony 11388-374	mg/L	2
Y, B1-5, O1-5, P1-5	Conductivity	Individual	7/31/2008; 8/4/2008	VWR Symphony 11388-372	µS/cm	1
Y, B1-5, O1-5, P1-5	Ammonia-Nitrogen	Individual	7/31/2008; 8/4/2008	LaMotte Kit 3304	mg/L	0.05
Y, B1-5, O1-5, P1-5	Nitrate	Individual	7/31/2008; 8/4/2008	LaMotte Kit 3319	mg/L	0.25
Y, B1-5, O1-5, P1-5	Nitrite	Individual	7/31/2008; 8/4/2008	LaMotte Kit 7674	mg/L	0.02
Y, B1-5, O1-5, P1-5	Hardness	Individual	7/31/2008; 8/4/2008	LaMotte Kit 4824-DR-LT	mg/L	20
Y, B1-5, O1-5, P1-5	Alkalinity	Individual	7/31/2008; 8/4/2008	LaMotte Kit 4419-DR	mg/L	20
Y, B1-5, O1-5, P1-5	Sulfate	Individual	7/31/2008; 8/4/2008	LaMotte Kit 7778	mg/L	20
Y, B1-5, O1-5, P1-5	Phosphate	Individual	7/31/2008; 8/4/2008	LaMotte Kit 7416-01	mg/L	0.05
Y, B1-5, O1-5, P1-5	Total Chlorine	Individual	7/31/2008; 8/4/2008	LaMotte Kit 6905	mg/L	0.2
Y, B1-5, O1-5, P1-5	DOC	Composite	7/31/2008; 8/4/2008	EPA 415.3	mg/L	0.01
Field						
Stock Solutions (B, O, P)	Cadmium	Stock	7/30/2008	EPA ILM05.2D	mg/L	0.0031
Stock Solutions (B, O, P)	Copper	Stock	7/30/2008	EPA ILM05.2D	mg/L	0.068
Stock Solutions (B, O, P)	Zinc	Stock	7/30/2008	EPA ILM05.2D	mg/L	0.18
Y, B1-5, O1-5, P1-5	Temperature	Individual	7/31/2008; 8/4/2008	Alcohol thermometer	˚C	0
Source Water	pH	Source	7/31/2008; 8/4/2008	VWR Symphony 14002-860	s.u.	0
Source Water	DO	Source	7/31/2008; 8/4/2008	VWR Symphony 11388-374	mg/L	2
Source Water	Conductivity	Source	7/31/2008; 8/4/2008	VWR Symphony 11388-372	µS/cm	1
Source Water	Ammonia-Nitrogen	Source	7/31/2008	LaMotte Kit 3304	mg/L	0.05

Table 1. Samples Collected, Analytical Methods and Associated Method Detection Limits

Treatment Groups	Parameter	Sample Type	Dates	Method	Unit	LOD
Field (continued)						
Source Water	Nitrate	Source	7/31/2008	LaMotte Kit 3319	mg/L	0.25
Source Water	Nitrite	Source	7/31/2008	LaMotte Kit 7674	mg/L	0.02
Source Water	Hardness	Source	7/31/2008	LaMotte Kit 4824-DR-LT	mg/L	20
Source Water	Alkalinity	Source	7/31/2008	LaMotte Kit 4419-DR	mg/L	20
Source Water	Sulfate	Source	7/31/2008	LaMotte Kit 7778	mg/L	20
Source Water	Phosphate	Source	7/31/2008	LaMotte Kit 7416-01	mg/L	0.05
Source Water	Total Chlorine	Source	7/31/2008	LaMotte Kit 6905	mg/L	0.2

Notes:

Composite - Composite sample of all replicates per treatment group measured

Individual - Measurement conducted in randomly selected test chamber

Stock - Metals analyzed in stock solutions; B – Zinc, 20,000 mg/L; O – Copper, 1,600 mg/L; P – Cadmium, 2,000 mg/L

DO – Dissolved oxygen

DOC – Dissolved organic carbon

LOD – Limit of detection

Table 2. Water Quality Measurements in Blanks

Sample Type	Parameter	Study	Date Analyzed	Method	Unit	Value
RO Water	DOC	Lab	7/30/2008	EPA 415.3	mg/L	0.22
Tap Water	DOC	Lab	7/30/2008	EPA 415.3	mg/L	2.98
UCR UFS Water (Background)	Cadmium	Field	7/25/ - 8/5/2008	EPA 415.3	mg/L	0.026-0.09
UCR UFS Water (Background)	Copper	Field	7/25/ - 8/5/2008	EPA 415.3	mg/L	0.72-0.88
UCR UFS Water (Background)	Zinc	Field	7/25/ - 8/5/2008	EPA 415.3	mg/L	8.8-22
Method Blank	Cadmium	Lab	7/31/2008; 8/4/2008	EPA ILM05.2D	mg/L	≤0.037
Method Blank	Cadmium	Field	7/30/2008	EPA ILM05.2D	mg/L	≤2.159
Method Blank	Copper	Lab	7/31/2008; 8/4/2008	EPA ILM05.2D	mg/L	<LOD
Method Blank	Copper	Field	7/30/2008	EPA ILM05.2D	mg/L	≤0.36
Method Blank	Zinc	Lab	7/31/2008; 8/4/2008	EPA ILM05.2D	mg/L	≤0.74
Method Blank	Zinc	Field	7/30/2008	EPA ILM05.2D	mg/L	<LOD

Notes:
DO – Dissolved oxygen
DOC – Dissolved organic carbon
LOD – Limit of detection
RO – Reverse osmosis
UCR – Upper Columbia River
UFS – Upstream field site

Table 3. Nominal and Measured (Mean ± Standard Deviation) Exposure Concentrations for Copper, Cadmium, and Zinc during the 96-hour Acute Exposures with White Sturgeon

Dissolved Metal	Treatment No.	River (µg/L)		Laboratory (µg/L)	
		Nominal	Estimated	Nominal	Measured
Copper	0	0	0.81	0	0.90 (0.13)
	1	1.56	2.1	1.56	1.9 (0.02)
	2	6.25	5.9	6.25	5.9 (0.11)
	3	25	21	25	22 (0.61)
	4	100	82	100	93 (3.9)
	5	400	327	400	382 (5.2)
Cadmium	0	0	0.07	0	0.08 (0.06)
	1	1.95	1.70	1.95	0.93 (1.2)
	2	7.81	6.60	7.81	7.6 (0.71)
	3	31.25	26	31.25	26 (2.6)
	4	125	105	125	107 (8.9)
	5	500	418	500	432 (26)
Zinc	0	0	16	0	15 (9.0)
	1	19.5	34	19.5	28 (8.4)
	2	78.1	88	78.1	76 (5.9)
	3	312.5	303	312.5	277 (3.8)
	4	1250	1164	1250	1284 (216)
	5	5000	4606	5000	4466 (40)

3.2 ROUTINE WATER QUALITY

Water quality measures recorded throughout the duration of the study are presented within Table 4. In short, averages (± standard deviation) for key water quality parameters were as follows:

- Water temperature = 16.7°C (± 0.20)
- DO = 75.9 percent (± 4.0)
- pH = 7.4 s.u. (± 0.07)
- Conductivity = 224 microSiemens per centimeter (µS/cm; ± 4.5)
- Hardness = 75.4 mg/L as calcium carbonate (± 1.9)
- Total ammonia nitrogen = <0.025 mg as N/L
- DOC = 2.75 mg/L (± 0.50).

Table 4. Summary of Mean ± Standard Deviation of Water Quality Parameters

Parameter	Cd Lab	Cu Lab	Zn Lab	Field[a]
Temperature (˚C)[b]	16.8 (± 0.1)	16.7 (± 0.21)	16.8 (± 0.09)	15.5 (± 1.2)
pH (s.u.)	7.4 (± 0.07)	7.4 (± 0.06)	7.3 (± 0.05)	7.8 (± 0.35)
DO (%)	77 (± 6.1)	78 (± 2.1)	76 (± 1.9)	82 (± 5.4)
Conductivity (µS/cm)	224 (± 5.8)	226 (± 2.5)	225 (± 5.1)	140 (± 0.35)
Ammonia Nitrogen (ppm)	<0.025*	<0.025*	<0.025*	<0.025*
Nitrate (ppm)	<0.25*	<0.25*	<0.25*	<0.25*
Nitrite (ppm)	<0.01*	<0.01*	<0.01*	<0.01*
Hardness (ppm)	76 (± 2.2)	75 (± 0.99)	76 (± 0.82)	72
Alkalinity (ppm)	50 (± 0.3)	50 (± 0.3)	50 (± 0.3)	62
Sulfate (ppm)	50 (± 0)	50 (± 0)	50 (± 0)	0
Phosphate (ppm)	<0.025*	<0.025*	<0.025*	nd
Total Chlorine (ppm)	<0.1*	<0.1*	<0.1*	0.1
DOC (mg/L)	2.5 (± 0.49)	2.6 (± 0.37)	2.6 (± 0.31)	nd

Notes:

[a] Measurement conducted in river water prior to dilution of stock solutions to avoid contamination of equipment (exception [b])
[b] Measured in randomly selected test chamber
Cd – Cadmium
Cu – Copper
DO – Dissolved Oxygen
DOC – Dissolved Organic Carbon
Zn - Zinc
Lab - Laboratory study
Field - Field study (see [a])
* Limit of Detection, all values below this value
nd - parameter not determined

3.3 MORTALITY

For each metal tested, concentration-dependent and statistically significant increases in mortality were observed at the highest concentrations (Table 5). With the exception of one fish in one of the five replicate treatments, no control mortalities were observed. No mortalities were observed in any treatments for which the two lowest dose concentrations were evaluated. Exposure to 21 and 22 µg/L copper resulted in 34 and 11 percent mortality in river and lab water, respectively. Low (4.4 percent) or no mortalities were observed for cadmium and zinc in the medium treatment groups. Greatest mortalities (>90 percent) occurred in the top two treatment groups of all metals tested. Relatively high mortalities were observed in the lab when sturgeon were exposed to 26 and 277 µg/L cadmium and zinc, respectively.

Table 5. Mean ± Standard Deviation Percent Mortality and Lethal Concentrations (LC50) for Early Life-stages of White Sturgeon after 96 hours of Exposure to Copper, Cadmium and Zinc

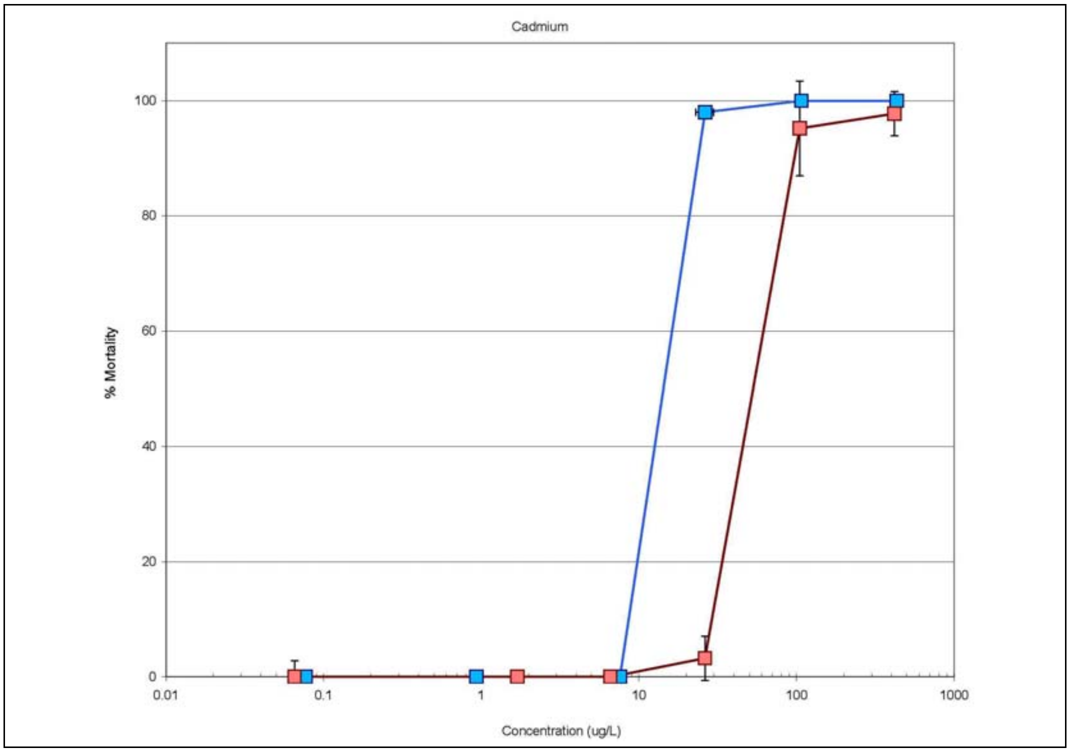
Treatment [a]	Cu (% Mortality)		Cd (% Mortality)		Zn (% Mortality)	
	River	Laboratory	River	Laboratory	River	Laboratory
0	1.3 (± 2.8)	0 (± 0)	1.3 (± 2.8)	0 (± 0)	1.3 (± 2.8)	0 (± 0)
1	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
2	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
3	34 (± 23)**	11 (± 14)	4.4 (± 3.9)	100 (± 0)***	0 (± 0)	100 (± 0)***
4	98 (± 3.9)***	93 (± 6.5)***	95 (± 8.3)***	100 (± 0)***	96 (± 3.7)***	100 (± 0)***
5	100 (± 0)***	100 (± 0)***	98 (± 3.9)***	100 (± 0)***	100 (± 0)***	100 (± 0)***
LC50 (µg/L)	44	30	14	53	145	612

Notes:
[a] Refer to Table 3 for a summary of the treatment numbers listed herein.
Cd – Cadmium
Cu – Copper
Zn – Zinc
Asterisks = significant mortality relative to controls as determined using the Bonferroni test; $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***)

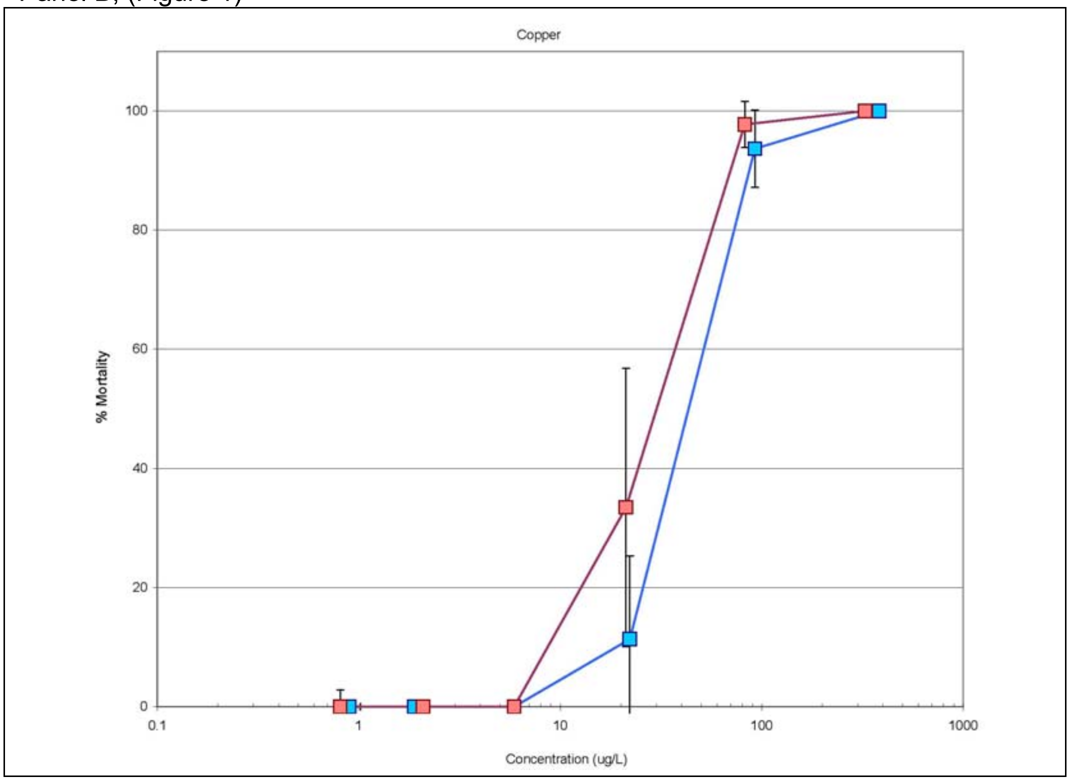
The biotic ligand model (BLM; Di Toro et al. 2001; Santore et al. 2001, 2002; HydroQual 2007) was calibrated using the metal concentrations (see Table 3) and associated toxicological responses (see Table 5) for cadmium, copper, and zinc effects to white sturgeon. The BLM is a predictive model that can explain how the chemistry of different exposure scenarios can affect metal toxicity. The purpose of this calibration is to develop parameter files that will allow the BLM to predict normalized acute effect concentrations for white sturgeon to cadmium, copper, and zinc, thereby allowing the BLM to translate these effects from laboratory conditions to a wider variety of exposure scenarios where factors such as pH, DOC, hardness, and alkalinity may vary.

To perform the calibration, the BLM was run with measured (pH, DOC, temperature, chloride, sulfate, hardness, alkalinity) or estimated (calcium, magnesium, potassium, sodium) water quality parameters. Parameters that were not measured were estimated from the average concentrations measured in water samples used in related studies (Entrix 2011). Concentration-response relationships between dissolved metal and observed mortality are shown for cadmium on Figure 1 (Panel A) or as normalized for bioavailability by considering accumulation on the biotic ligand (Figure 2). Similar figures are shown for dissolved and biotic-ligand bound copper (Figure 1 [Panel B] and Figure 3) and zinc (Figure 1 [Panel C] and Figure 4). From the concentration-response on the biotic ligand, the critical accumulation associated with 50 percent mortality (LA50) can be estimated, and the value for each metal is shown on in the top left corner of Figures 2, 3, and 4. Similar BLM normalization for field responses shown in Figure 1 cannot be performed at this time due to the lack of data (i.e., DOC concentration).

Panel A, (Figure 1)



Panel B, (Figure 1)



Panel C (Figure 1)

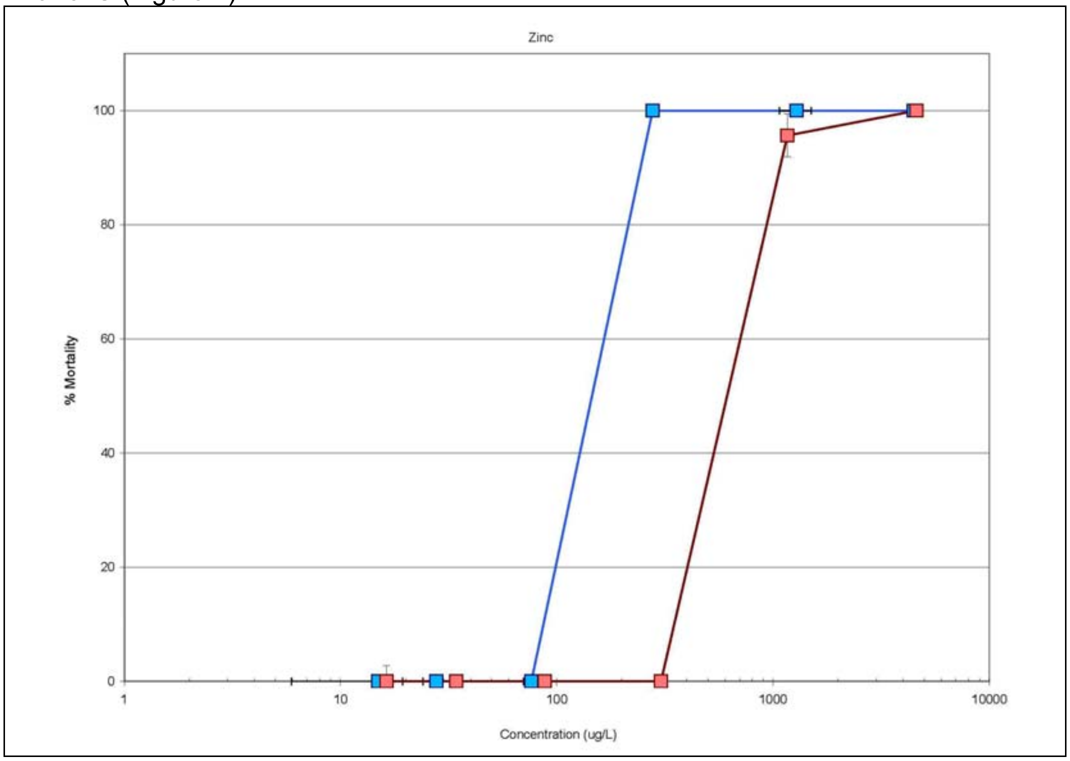


Figure 1. Average Percent Mortality of White Sturgeon Fry Exposed from 8 through 12 dph to Cadmium (Panel A), Copper (Panel B), and Zinc (Panel C) Under Static Renewal Conditions in Laboratory (blue) and River (red) Water, Respectively. Note: Exposure concentration represents measured concentrations of metals. Error bars = 1 SD

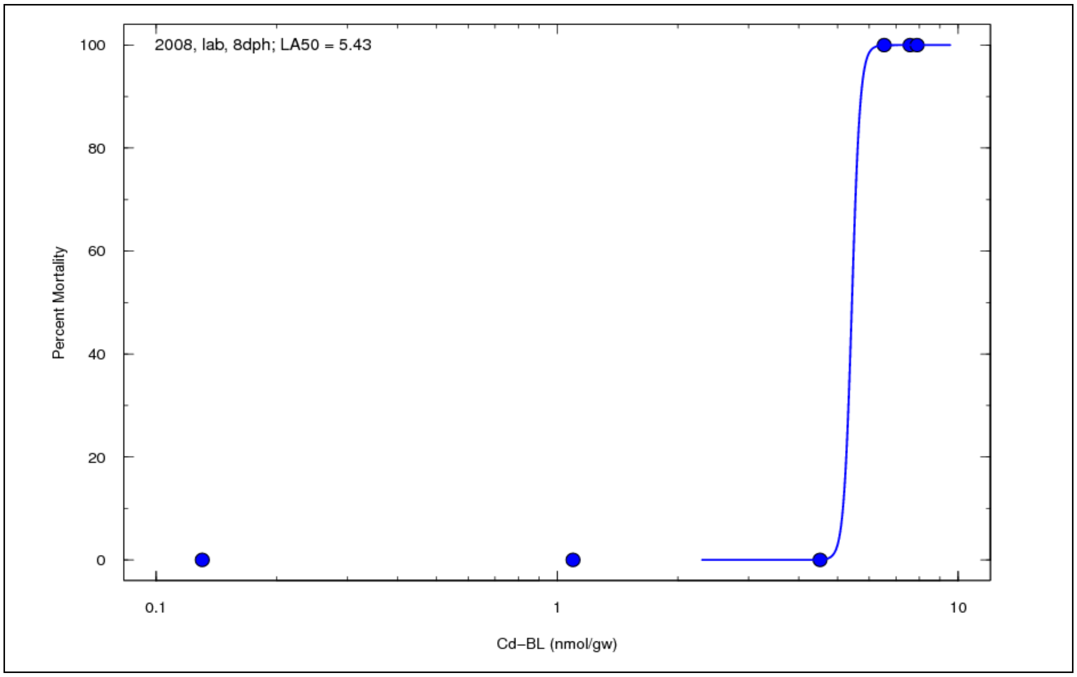


Figure 2. Concentration-response Relationship for Cadmium in 96-hour Exposures of White Sturgeon; Based on BLM Predicted Concentration of Accumulated Cadmium at Biotic Ligand Sites.

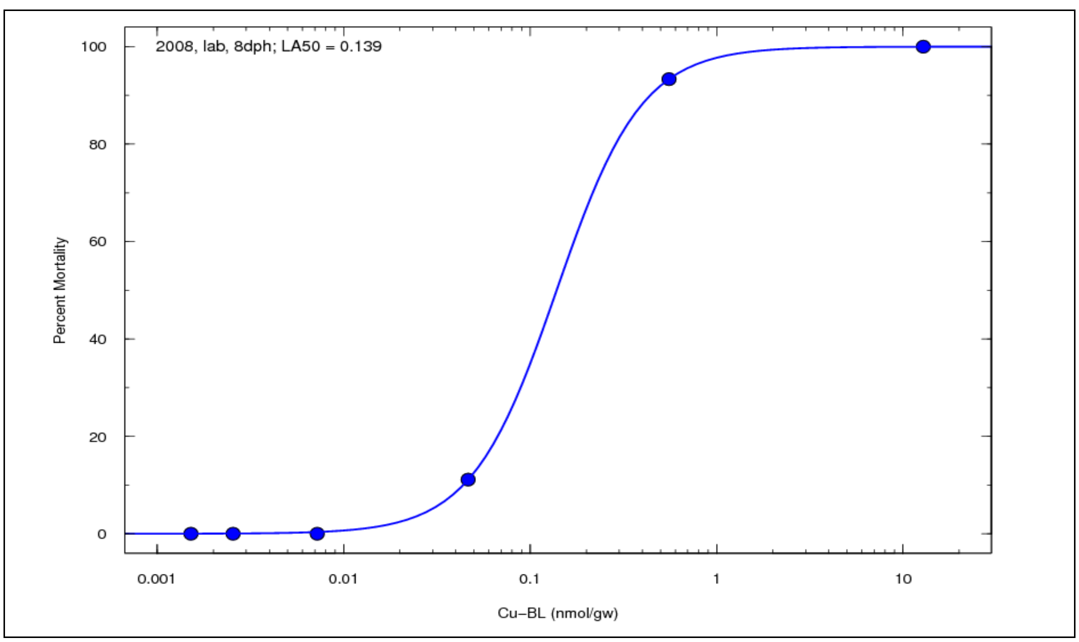


Figure 3. Concentration-response Relationship for Copper in 96-hour Exposures of White Sturgeon; Based on BLM Predicted Concentration of Accumulated Copper at Biotic Ligand Sites.

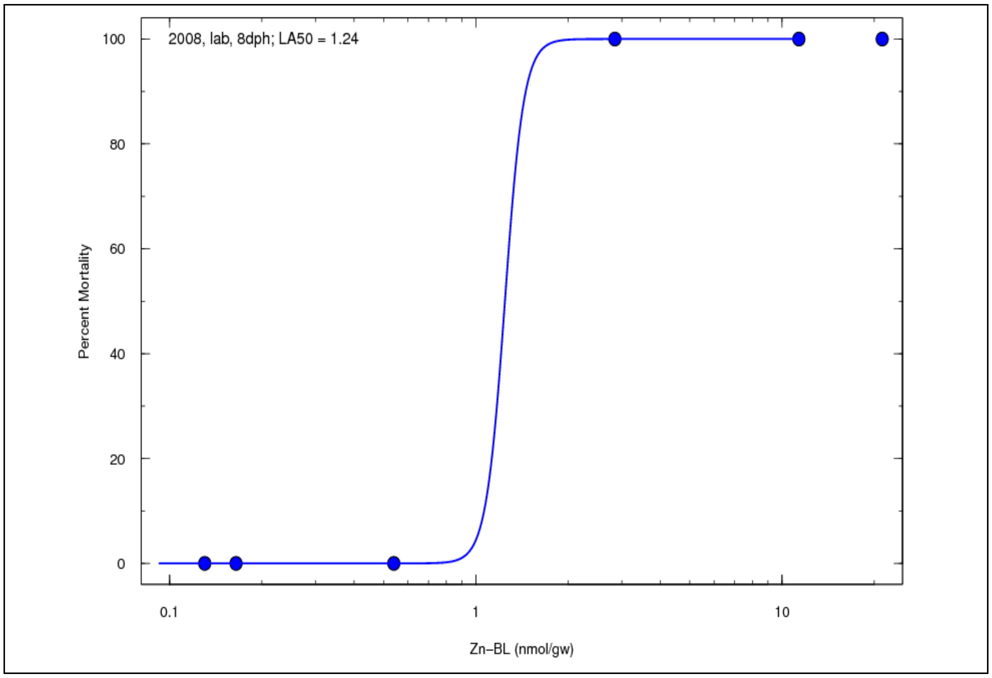


Figure 4. Concentration-response Relationship for Zinc in 96-Hour Exposures of White Sturgeon; Based on BLM Predicted Concentration of Accumulated Zinc at Biotic Ligand Sites.

Values for acute water quality criteria for the state of Washington and for EPA are shown in Table 6. For each of these metals, both the state and EPA acute water quality criteria are considerably lower than the acute LC50 for sturgeon (Table 5), indicating that the acute criteria for cadmium, copper, and zinc are protective for acute exposures of these metals to sturgeon.

Table 6. Acute Water Quality Criteria for Dissolved Metals in the State of Washington (WA) (WAC 173-201A-240) and by EPA

Dissolved Metal	Average hardness	Acute criterion (µg/L) - WA[a]	Acute criterion (µg/L) - EPA[b]
Cadmium	76 mg/L as $CaCO_3$	2.8	3.2
Copper	74.4 mg/L as $CaCO_3$	12.9	9.2
Zinc	75.7 mg/L as $CaCO_3$	90.4	92.6

Notes:

[a] Acute criteria for the state of WA are calculated based on an average water hardness using the following equations:

$\text{Cd WQC} = 0.955(e^{(1.128[\ln(\text{hardness})]-3.828)})$

$\text{Cu WQC} = 0.960(e^{(0.9422[\ln(\text{hardness})] - 1.464)})$

$\text{Zn WQC} = 0.978(e^{(0.8473[\ln(\text{hardness})] + 0.8604)})$

[b] Acute criteria for EPA are calculated based on an average water hardness (Cd and Zn) using the following equations, or on pH, DOC and major ions using the BLM (Cu):

$\text{Cd WQC} = 0.955(e^{(1.128[\ln(\text{hardness})]-3.6867)})$

Cu WQC is based on the BLM (USEPA 2007).

$\text{Zn WQC} = 0.978(e^{(0.8473[\ln(\text{hardness})] + 0.884)})$

4 CONCLUSIONS

Acute exposures of cadmium, copper and zinc to ELS of white sturgeon (*A. transmontanus*) resulted in acute toxicity (LC50s) at concentrations of 14, 44, and 145 µg/L respectively in laboratory water, and concentrations of 53, 30, and 612 µg/L in river water. In all cases, these values are substantially greater than the acute water quality criteria for these metals in the state of Washington, which correspond to 2.8, 8.8, 90.4 µg/L for cadmium, copper, and zinc respectively, thereby indicating that acute criteria are protective for acute exposures of cadmium, copper, and zinc to white sturgeon.

BLM calibrations were performed for these exposures and concentration-response relationships on the biotic ligand suggest that accumulation on the biotic ligand can be correlated with effects. These concentration-response relationships were used to derive critical accumulation levels (LA50s) that can be used in parameter files that will allow application of the BLM to predict how acute effects for these metals to white sturgeon will change with changing water chemistry.

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