UPPER COLUMBIA RIVER
REMEDIAL INVESTIGATION AND FEASIBILITY STUDY

Quality Assurance Plan for the Assessment of Surface Water Toxicity to White Sturgeon (*Acipenser transmontanus*) in the Upper Columbia River

Prepared for
Teck American Incorporated
P.O. Box 3087
Spokane, Washington 99220, USA

Prepared by
ENTRIX, Inc.

Saskatoon, SK S7N 3B5, Canada

and

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon, SK S7N 3B5, Canada

in consultation with
Cardwell Consulting LLC

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SECTION A: PROJECT MANAGEMENT

A1 TITLE AND APPROVAL SHEET

QUALITY ASSURANCE PROJECT PLAN FOR THE "ASSESSMENT OF SURFACE WATER TOXICITY TO WHITE STURGEON (*ACIPENSER TRANSMONTANUS*) IN THE UPPER COLUMBIA RIVER" TO BE CONDUCTED IN 2009

Quality Assurance Project Plan Approvals

Teck Project Coordinator:	Marko Adzic		Date: 06/27/09
Teck Technical Team Coordinator & Task Manager:	Markus Hecker		Date: May-1'09
U of S Principal Investigator:	John Giesy		Date: May-1'09
Task QA Coordinator:	Shaun Roark		Date:
Chemical Laboratory Project Manager:	Jeff Christian		Date: 6/29/09
Chemical Laboratory QA Manager:	Julie Gish		Date: 6-29-09

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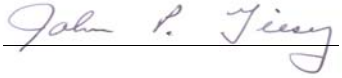
U of S Principal Investigator:	John Giesy		Date: May-1'09
Task QA Coordinator:	Shaun Roark	_____	Date: _____
Chemical Laboratory Project Manager:	Jeff Christian	_____	Date: _____
Chemical Laboratory QA Manager:	Julie Gish	_____	Date: _____

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ACRONYMS AND ABBREVIATIONS

Agreement	June 2, 2006, Settlement Agreement
ASTM	American Society for Testing and Materials
AWQC	ambient water quality criterion
BERA	baseline ecological risk assessment
CCC	chronic continuous criterion
ChE	cholinesterase
COC	chain-of-custody
COI	chemical of interest
CSM	conceptual site model
CSOIII	Combined Sewer Outfall III
DBMS	database management system
DOC	dissolved organic carbon
dph	days post hatch
DQO	data quality objective
Ecology	Washington State Department of Ecology
EDD	electronic data deliverable
ELS	early life stages
ENTRIX	ENTRIX, Inc.
EPA	U.S. Environmental Protection Agency
ERB	equipment rinsate blank
ICP/AES	inductively coupled plasma/atomic emission spectrometry
ICP/MS	inductively coupled plasma/mass spectrometry
Kow	octanol–water partition coefficient
Lake Roosevelt	Franklin D. Roosevelt Lake
LC50	median lethal concentration
LCS	laboratory control sample
LCSD	laboratory control sample duplicate
LOAEC	lowest-observed-adverse-effect concentration

MRL	method reporting limit
MS/MSD	matrix spike/matrix spike duplicate
NOAEC	no-observed-adverse-effect concentration
PARCCS	precision, accuracy, representativeness, comparability, completeness, and sensitivity
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PDA	personal digital assistant
QA/QC	quality assurance and quality control
QAPP	quality assurance project plan
RI/FS	remedial investigation and feasibility study
RM	river mile
RPD	relative percent difference
Site	Upper Columbia River site
SLERA	screening level ecological risk assessment
SM	*Standard Methods for the Examination of Water and Wastewater*
SOP	standard operating procedure
SRM	standard reference material
SRMD	standard reference material duplicate
T&E	threatened and endangered
TDS	total dissolved solids
Teck	Teck American Incorporated
TOC	total organic carbon
TSS	total suspended solids
U of S	University of Saskatchewan
UCR	Upper Columbia River
USFWS	U.S. Fish & Wildlife Service
USGS	U.S. Geological Survey
WER	water effects ratio

UNITS OF MEASURE

°C	degrees Celsius
L	liter(s)
mg	milligram(s)
mL	milliliter(s)
µg	microgram(s)

A3 DISTRIBUTION LIST

Teck Project Coordinator:	Marko Adzic
Teck Technical Team Coordinator & Task Manager:	Markus Hecker
U of S Principal Investigator:	John Giesy
Task QA Coordinator:	Shaun Roark
Study Team Leader:	David Vardy
Database Administrator:	Dreas Nielsen
Chemical Laboratory Project Manager:	To be determined
Chemical Laboratory QA Manager:	To be determined

A4 INTRODUCTION AND TASK ORGANIZATION

A4.1 Introduction

This document presents the approach and rationale for conducting a study to assess surface water toxicity to white sturgeon (*Acipenser transmontanus*) at early life stages (ELS) in the Upper Columbia River (UCR) site (Site)[1] and selected upstream areas in support of the remedial investigation and feasibility study (RI/FS) and in particular to be used in the baseline ecological risk assessment (BERA) for the Site. As noted in the RI/FS work plan (TAI 2009a), the primary objectives of the RI/FS are to investigate the nature and extent of contamination at the Site, to provide information to support baseline risk assessments for human health (to be completed by the U.S. Environmental Protection Agency [EPA]) and the environment (to be completed by Teck American Incorporated [Teck]), and to develop and evaluate potential remedial alternatives for the Site.

One of the key species of interest at the Site is white sturgeon for which an ELS-specific recruitment bottleneck had been reported in recent years (Hildebrand et al. 1999; R.L. & L. Environmental Services Ltd. 1994, 1995, 1996). As indicated within the preliminary conceptual site model (CSM; Figure A4-1) presented in the RI/FS work plan (TAI 2009a), three potential exposure pathways for white sturgeon and other bottom dwelling fish have been identified (TCAI 2008). These include:

- Water: Contact/uptake/gill uptake of surface water or sediment pore water
- Sediment: Contact/uptake or ingestion of sediments from the thalweg or near shore
- Diet: Ingestion of aquatic biota.

The 2009 studies of surface water toxicity to ELS of white sturgeon (hereinafter referred to as "2009 sturgeon ELS studies") will evaluate if there are unacceptable risks (i.e., potential toxicity) of surface water close to the sediment–water interface (hereinafter referred to as near-bottom water) in the UCR to ELS of white sturgeon. Potential issues related to other exposure routes such as sediment and possibly diet will be addressed in future studies, and will not be referred to further in this document.

[1] The UCR site as defined within the June 2, 2006, Settlement Agreement is the areal extent of hazardous substances contamination within the United States in or adjacent to the Upper Columbia River, including the Franklin D. Roosevelt Lake, from the U.S.-Canadian border to the Grand Coulee Dam, and those areas in proximity to the contamination which are suitable and necessary for implementation of response actions.

This document presents the scientific rationale and general approach for the conduct of studies to determine the potential toxicity of near-bottom water to ELS of white sturgeon within the UCR. Available existing data collected during previous years will be used to aid in the development of this quality assurance project plan (QAPP) (see Section A5). The data quality objectives (DQOs) presented herein were developed to address the needs of the UCR RI/FS in accordance with USEPA (2006a) (see Section A7).

A4.2 Task Organization

This section presents the organizational structure for activities associated with the 2009 sturgeon ELS studies, including task management and oversight, fieldwork, sample analysis, and data management. ENTRIX, Inc. (ENTRIX), and the Environmental Toxicology Group at the University of Saskatchewan (U of S) are conducting this work with oversight from EPA and with consultation from the Teck technical team. The combined roles of the U of S and ENTRIX are presented in Figure A4-2.

A4.2.1 EPA Organization and Responsibilities

EPA will oversee Teck activities associated with the 2009 sturgeon ELS studies, and will coordinate U.S. Department of the Interior, Washington State Department of Ecology (Ecology), and tribal (i.e., the Confederated Tribes of the Colville Reservation and the Spokane Tribe of Indians) input with respect to the review of technical documents submitted by Teck. The project coordinator for EPA is Kevin Rochlin. Mr. Rochlin will also be responsible for ensuring that the work performed is consistent with all applicable EPA guidance. The EPA QA manager will be assigned by EPA.

A4.2.2 Teck Organization and Responsibilities

With the support of ENTRIX and the U of S—in consultation with the Teck technical team—Teck is responsible for conducting the 2009 sturgeon ELS studies with oversight provided by EPA. Mr. Marko Adzic will serve as Teck's project manager and will have the primary responsibility for ensuring that Teck meets all the requirements and associated deliverables specified within the June 2, 2006, Settlement Agreement (Agreement) (USEPA 2006c). Dr. Markus Hecker will be responsible for overseeing all technical aspects of this task, coordinating with the EPA technical team, and managing the overall task schedule.

A4.2.3 Key Task Personnel

Task Manager—Dr. Markus Hecker (ENTRIX; U of S) will oversee and approve all task activities; review quality assurance reports; approve final project quality assurance

needs; and act as liaison between the U of S principal investigator and other U of S personnel, Teck personnel, the Teck technical team, and the Teck project manager (Marko Adzic). Dr. Hecker will be responsible for compiling summary results and project final reports. He will also be responsible for statistical analysis and data interpretation.

U of S Principal Investigator—Prof. John P. Giesy (U of S) will advise the project manager in overseeing and approving all project activities; review quality assurance reports; approve final project quality assurance needs; authorize necessary actions and adjustments related to U of S activities to accomplish program quality assurance objectives; and act as liaison between agencies, staff, and the project manager.

Quality Assurance Auditor—(To be named) An independent external advisor will review all quality assurance activities to ensure compliance with contract specifications. The auditor will review all data deliverables to ensure data quality and usability. The identity of this person or persons will be determined by discussion with the Teck project manager.

Study Team Leader—David Vardy, under the supervision of Markus Hecker (ENTRIX/U of S), will oversee all research activities and supervise the study crews. The study team leader will ensure that proper sample collection, preservation, storage, transport, and chain-of-custody (COC) procedures are followed, will inform the project quality assurance manager when problems occur, and will communicate and document corrective actions taken. The study team leader will discuss study activities with the project manager.

Analytical Chemistry Laboratory Coordinator—(To be named) The analytical chemistry laboratory coordinator is responsible for ensuring that laboratory method development is satisfactorily completed prior to the analysis of samples collected for this task; coordinating with the testing laboratory and tracking the laboratory's progress; verifying that the laboratory has implemented the requirements of this QAPP; addressing quality assurance issues related to the laboratory analyses; ensuring that laboratory capacity is sufficient to undertake the required analyses in a timely manner; and addressing scheduling issues related to laboratory analyses. The chemistry laboratory coordinator will report directly to the task manager and will work closely with the study team leader and the quality assurance manager.

Quality Assurance Manager—Dr. Shaun Roark (ENTRIX) will initiate audits on work completed by project personnel. The quality assurance manager will review program quality assurance activities, quality problems, and quality-related requests. In response

to experimental and bioanalytical findings, the quality assurance manager will approve corrective actions. The quality assurance manager will report quality non-conformances to the Project Manager and review all pertinent portions of the both U of S and ENTRIX deliverables before they are transmitted to ensure conformance with quality assurance and quality control (QA/QC) procedures and quality work products.

Data Manager—Dreas Nielsen (Integral) is the database administrator and will have primary responsibility for incorporating the results of the 2009 sturgeon ELS studies into the project database, including establishment of storage formats and standards for coding of data. Dr. Markus Hecker (ENTRIX) will coordinate ENTRIX data acquisition and storage during the execution of the studies. He will be responsible for the structure and operation of ENTRIX's working databases, and will coordinate with Mr. Nielsen on the establishment of formats for storage and transmittal of information to the project database.

A4.2.4 Analytical Contract Laboratory

The following responsibilities apply to the project managers and quality assurance manager at the analytical laboratory used for the analysis of water and potential other samples to be collected during the 2009 sturgeon studies. The laboratory will be selected prior to initiation of fieldwork. It is possible that more than one laboratory will be selected to meet analytical goals.

Laboratory Project Manager—The laboratory project manager is responsible for the successful and timely completion of sample analyses, as well as the following actions:

- Ensure that samples are received and logged correctly, that the correct methods and modifications are used, and that data are reported within specified turnaround times
- Review analytical data to ensure that procedures were followed as required in this QAPP, the cited methods, and laboratory standard operating procedures (SOPs)
- Apprise the chemical laboratory coordinator of the schedule and status of sample analyses and data package preparation
- Notify the chemical laboratory coordinator if problems occur in sample receiving, analysis, or scheduling, or if control limits cannot be met
- Take appropriate corrective action as necessary

- Report data and supporting quality assurance information as specified in this QAPP.

Laboratory Quality Assurance Manager—The laboratory quality assurance manager is responsible for overseeing the quality assurance activities in the laboratory and ensuring the quality of the data for this task. Specific responsibilities include the following:

- Oversee and implement the laboratory's quality assurance program
- Maintain quality assurance records for each laboratory production unit
- Ensure that QA/QC procedures are implemented as required for each method and provide oversight of QA/QC practices and procedures
- Review and address or approve non-conformity and corrective action reports
- Coordinate responses to any quality control issues that affect this task with the laboratory project manager.

A4.3 Problem Definition and Background

There are concerns about the potential contribution of chemicals of interest (COIs) in the UCR to the poor recruitment of white sturgeon in the Columbia River between Grand Coulee Dam in the U.S. and the Hugh L. Keenlyside Dam in Canada that has been documented since the 1970s. COIs in surface water of the UCR were previously identified in the UCR draft screening level ecological risk assessment (SLERA) (TCAI 2008). In this section, information on existing data on the potential toxicity of COIs to white sturgeon and related species is reviewed.

A4.3.1 Conceptual Site Model for Sturgeon

The general CSM provided in Section A4.1 has been simplified and more specifically targeted to white sturgeon and other benthic feeding fish species in the UCR to address the specific needs for the assessment of potential toxicity of COIs in the UCR to these species (Figure A4-3). This CSM shows that chemicals in the UCR may occur in dissolved, suspended, and settleable particulate phases. In surface water and sediment pore waters, both metals and organic chemicals will partition between dissolved, particulate, and biotic phases. Bioaccumulation occurs via uptake from water and diet. In the case of neutral organic chemicals, uptake occurs via passive processes (Gobas and Mackay 1987), whereas for metals it may occur via both passive and active transport processes (Grosell et al. 2002, 2007). Partitioning between the phases is chemical- and site-specific (Ankley et al. 1996; Di Toro et al. 1991, 2001; USEPA 2005b), and

bioaccumulation varies by chemical, the physicochemical characteristics of each location, and the species and life stage of the organism.

In general, complete metal exposure pathways for all life stages of white sturgeon include uptake from surface water and direct contact with sediment (including porewater during earlier life-stages). In addition, dietary (food web) exposures are complete for juvenile and adult fish. Although a potentially complete exposure pathway, maternal transfer of metals to embryos is not likely (McKim and Benoit 1971; Holcombe et al. 1976, 1979). Metals bound to suspended sediments constitute a complete exposure pathway for filter-feeding prey of fish, but not for the omnivorous sturgeon, cottids, and suckers.

Due to the extreme hydrophobicity of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), water exposures of these chemicals to fish generally are insignificant compared to dietary exposures (Bruggeman et al. 1984). For the purpose of the studies discussed here to assess the potential toxicity related to exposure to COIs in near-bottom water, therefore, hydrophobic chemicals such as the above discussed hydroaromatic hydrocarbons are not further considered in this QAPP.

A4.4 Relative Sensitivity of Sturgeon to Selected Chemicals

A search of the scientific literature has indicated that some species of sturgeon (family Acipenseridae) are more sensitive to some chemicals, including selected COIs such as copper, relative to other fish species, based on a number of laboratory studies. EPA's AQUIRE database (USEPA 2005a) was searched to determine the availability of toxicity data for three groups of fish that live and feed on the bottom of the UCR: white sturgeon, whitefish (family Salmonidae), and suckers (family Catostomidae). The objective of these literature searches was to assess whether these or closely related species are uniquely sensitive to chemicals compared to other species. Because none of these sturgeon species are used routinely in aquatic toxicity testing, the search focused on any chemical in which these and other species had been tested.

The AQUIRE database contains toxicity data for six sturgeon species in the genus *Acipenser*: *A. baerii* (Siberian sturgeon), *A. fulvescens* (lake sturgeon), *A. gueldenstaedtii* (Russian sturgeon), *A. ruthenis* (sterlet), *A. stellatus* (starry sturgeon), and *A. transmontanus* (white sturgeon). Most of the toxicity data available for these species are from relatively nonstandard endpoints and durations, which makes comparisons of sensitivity relative to other aquatic biota difficult. The following provide indications of

acute sensitivities of sturgeon relative to several threatened and endangered species of fish, and to fish and aquatic invertebrates in general, for several chemicals.

Dwyer et al. (2005) evaluated the acute sensitivities of several threatened and endangered (T&E) species, including Atlantic sturgeon (*A. oxyrhynchus*), shortnose sturgeon (*A. brevirostrum*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*), relative to the commonly tested rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), and sheepshead minnow (*Cyprinodon variegatus*). The chemicals tested were carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin, each representing different modes of toxic action (Figures A4-4 to A4-8). Of the T&E species tested—plus rainbow trout, fathead minnow and sheepshead minnow—the Atlantic sturgeon and shortnose sturgeon were the most and second most sensitive species, respectively. The relative sensitivities of sturgeon species as a whole varied greatly. For copper, 96-hour median lethal concentrations (LC50s) for the Atlantic sturgeon and shortnose sturgeon were 60 and 80 μg/L, respectively, similar to LC50s (70–80 μg/L) for several salmonid species. To further understand the sensitivity of sturgeon relative to a variety of other species, the toxicity values for sturgeon and other species from Dwyer et al. (2005) were plotted as species sensitivity distributions (Posthuma et al. 2002) based on toxicity data compiled from EPA's AQUIRE database. As shown, for 4–nonylphenol, pentachlorophenol, and permethrin in particular, some sturgeon species may be among the more acutely sensitive fish species compared to the group of test species investigated by Dwyer et al. (2005).

Toxicity data for mountain whitefish (*P. williamsoni*) in the AQUIRE database are limited to three acute toxicity values for ammonia, which were incorporated into EPA's ambient water quality criterion (AWQC) for ammonia (USEPA 1999). The genus *Prosopium* was the most acutely sensitive freshwater genus to ammonia and the species *P. williamsoni* was the second most acutely sensitive freshwater species to ammonia. Although this suggests that mountain whitefish may be a sensitive species to chemicals in general, it is important to note that species mean acute values for fish exposed to ammonia occupied a narrow range, from 11.23 μg N/L to 51.73 μg N/L (n=29 species) (USEPA 1999). The AQUIRE database contains some toxicity data for the lake whitefish (*Coregonus clupeaformis*), which is a different genus from mountain whitefish, but in the same family (Salmonidae), and occurs in lacustrine reaches of Franklin D. Roosevelt Lake (Lake Roosevelt).

The AQUIRE database does not include any toxicity data for the largescale sucker (*C. macrocheilus*), although toxicity data for several chemicals are available for the closely

related white sucker (*C. commersoni*). In the United States, white suckers are the most widely tested of the two *Catostomus* species, and because species within a genus—in this case *Catostomus*—are known to be more closely related in sensitivity than genera within a family or families within an order (Suter et al. 1983), the white sucker data may be indicative of the sensitivity of the *Catostomus* species inhabiting the UCR. The white sucker does not appear to be exceptionally acutely sensitive to copper or zinc, with 96-hour LC50 values of 886 μg/L for copper (test water hardness not reported) and 2,200 μg/L for zinc at a test water hardness of 18 mg/L. In EPA's fairly recent AWQC document for cadmium (USEPA 2001), the *Catostomus* genus ranks 41st of 55 genera for cadmium, suggesting its sensitivity is lower than the majority of fish species. For another point of comparison, the endangered razorback sucker *(Xyrauchen texanus*), although in a different genus than the largescale sucker but within the same family (Catostomidae), does not appear to be uniquely acutely sensitive to chemicals.

A5 EXISTING DATA

The available toxicity data for the exposure of ELS of white sturgeon to COIs or to matrices from the Site is limited. In this section, we summarize the available information regarding the potential toxicity of COIs to sturgeon and critique its utility for the UCR RI/FS. Information of appropriate quality is then used to develop the DQOs and to design the 2009 sturgeon studies.

A5.1 Historical Data—Toxicity Studies with White Sturgeon and Other Acipenseridae Species

Two studies have been conducted to assess the potential effects of liquid effluent from Teck's Trail, B.C., smelter to ELS of white sturgeon (Bruno 2004; ENTRIX 2007, unpublished). In addition, a series of studies have measured concentrations of selected COIs in tissues and malformations in sturgeon from the Columbia River between Hugh Keenlyside Dam and the U.S.-Canadian border (Kruse and Webb 2006). Finally, few studies have assessed the exposure of white and other sturgeon species to certain COIs under both under controlled laboratory conditions and in the field (Lapirova et al. 2000; Kruse and Scarneccia 2002a,b; Dwyer et al. 2005; USFWS and USGS 2008; ENTRIX, in prep.).

A5.1.1 Kruse and Webb (2006)

There have been several site-specific studies of the potential effects of metals to fish in the upper Columbia River. A comprehensive set of studies was conducted in the stretch

of the Columbia River between Hugh Keenlyside Dam and the U.S.-Canadian border in the early 1990s using mountain whitefish (*Prosopium williamsoni*) as the sentinel, or indicator, species (e.g., Antcliffe et al. 1997; Boyle 1992; Nener 1995). These studies failed to implicate concentrations of chemicals in surface water and in tissues of whitefish or their prey as being responsible for lesions and other measures of health effects in whitefish. However, given the different ecological niche that whitefish occupy compared to white sturgeon (opportunistic vs. strictly benthic), it can be assumed that these species may be subjected to different exposure pathways with respect to UCR COIs. Thus, the data obtained for whitefish in the above discussed studies are of only limited value with regard to assessment of potential risks related to exposure of white sturgeon in the UCR. A later set of studies attempted to evaluate the toxicity of environmental contaminants, including metals, to white sturgeon in the Canadian reach of the Columbia River between Hugh Keenlyside Dam and the U.S. border, with some of the recapture assessments having been conducted in the U.S. (Kruse and Webb 2006). These studies, however, did not find conclusive cause-and-effect relationships between any exposures to COIs and the incidences of effects observed.

A5.1.2 Bruno et al. (2004)

In support of the Upper Columbia River White Sturgeon Recovery Initiative, studies were conducted during summer/fall of 2002 to assess the toxicity of two effluents on ELS of white sturgeon. The matrices tested were Combined Sewer Outfall III (CSOIII) effluent from Teck's Trail smelter and effluent from the secondary foam tank of Celgar Pulp Company Ltd in Castlegar. The duration of the study was from 11–14 days post hatch (dph) through 61–64 dph. One hundred percent mortality was observed in the 50 and 100 percent CSOIII treatment groups after 17 and 5 days of exposure, respectively. No increased mortality relative to the controls occurred at 1 percent CSOIII concentration or in any of the two pulp mill exposure groups (1 and 100 percent effluent concentration). Control mortalities were 38.4 ± 28.4 percent (mean ± SD). No effects on growth or behavior were observed for any of the treatment groups, but it was concluded that the ability to detect such alterations in the 50 and 100 percent exposure groups were limited due to the short time until 100 percent mortality occurred.

There were a number of uncertainties associated with the Bruno (2004) work, which render these studies of limited use for the assessment of risks associated with COIs in the UCR. These include the limited exposure period that did not include evaluations of potential effects on possibly more sensitive life stages, including *in ovo* (e.g., eggs, embryos) and immediate post hatch effects. Furthermore, mortalities in the controls

were highly variable (average = 40 percent; coefficient of variation = 74 percent), which limits the utility of the data for risk assessment purposes given the great uncertainty related to the controls. Also, testing of effluent does not account for any possible co-factors present in river water (e.g., other contaminants and properties that influence bioavailability) that may interact with the COIs in effluent (test solution dilutions were done using laboratory water)..

A5.1.3 ENTRIX (2008, unpublished)—CSOIII Effluent Pilot Study

During summer 2007, ENTRIX, in cooperation with the U of S, conducted a study on Teck's Trail operations site to establish an exposure system that permits the assessment of the effects of toxicants and other factors on ELS of white sturgeon including *in ovo* exposure, embryo mortality/hatchability, and growth or survival effects of fry and juveniles in subchronic settings. During the pilot study, fertilized sturgeon eggs (7–10 days post fertilization) and 14-day-old fry were exposed to 25, 5, and 1 percent CSOIII effluent or a filtered tap water control for 38 days with the least concentration (1 percent effluent) representing a worst case scenario for dilution of the effluent in the river (assumed dilution > 1,000-fold = 0.1 percent). Concentrations of 18 metals that were analyzed did not differ significantly among treatments, and exceeded chronic EPA water quality criteria in two cases (lead and cadmium) and by a small margin. The pilot study revealed greater than 80 percent hatchability regardless of treatment group, which is in accordance or exceeds hatching rates that were reported by other hatcheries (see Section A5.2.1). Furthermore, survival in all exposure groups was significantly greater than that in the controls, which was attributed to the greater presence of epiphyton in these groups compared to the filtered tap water control (Figure A5-1). Although small, there were statistically significant differences in lengths of fry between the two greatest effluent treatment groups and the controls at the end of the study. Fish in the controls were slightly longer than those in the effluent groups. No differences among treatment groups were observed for weight. The maximum difference in lengths was observed between the negative controls and the 25 percent effluent treatment group (6 percent). Reasons for these differences in length were unclear.

Data collected during the pilot study indicates that there was no significant effect on survival of sturgeon fry associated with liquid CSOIII effluent of Teck's Trail facility tested during summer 2007. However, considering that the only matrix analyzed in this study was liquid effluent diluted with laboratory water, this precludes the results from being used for the assessment of near-bottom water toxicity to white sturgeon in the Columbia River as part of the UCR RI/FS. Based on the data and experiences collected

during these studies, the exposure design established for the testing of toxicity of liquid matrices to white sturgeon has been optimized and protocols and quality criteria have been developed for use in the 2009 studies in support of the risk assessment of COIs in the UCR to ELS of white sturgeon.

A5.1.4 Lapirova et al. (2000)

Lapirova et al. (2000) investigated the effects of copper, cadmium, and mercury on 2-month-old Siberian sturgeon (*Acipenser baeri*). The following LC50s were reported after an exposure period of 96 hours: 0.3 mg/L for cadmium; 0.15 mg/L for copper; and 0.03 mg/L for mercury. The data imply this species is more sensitive to exposure to copper than to cadmium, which is contrary to observations made for other fish species (e.g., Besser et al. 2007). However, there are a number of uncertainties that limit the use of data from this study for the assessment of risks associated with copper, cadmium, or mercury concentrations in the UCR. First, Lapirova et al. (2000) tested a different species of sturgeon, and it has been shown that different sturgeon species can differ substantially in their sensitivity to metals such as copper (Dwyer et al. 2005). Second, this study tested 2-month-old juveniles, and thus, there are uncertainties in the applicability of these data to the sensitivity of earlier life-stages (embryo and fry) to copper. Finally, the Lapirova et al. (2000) study was of an acute nature (96-hour LC50 study), which limits the utilization of the data for predictions to the chronic environmental exposure scenarios.

A5.1.5 Kruse and Scarneccia (2002a)

Kruse and Scarneccia (2002a) conducted a field study in the Kootenay River, B.C., to assess the potential effect of environmental mixtures of metals and organochlorine chemicals to white sturgeon. The authors measured concentrations of selected chemicals and elements in different tissues (ovary, testis, juvenile whole body) and attempted to correlate these with a series of biological endpoints including plasma sex steroid production, plasma vitellogenin concentrations, egg size/production, DNA chromosomal variability, liver histology, and cholinesterase (ChE) activities. Kruse and Scarneccia (2002a) reported some correlations between individual contaminants measured in tissues and selected biological endpoints such as plasma sex steroid concentrations, chromosomal DNA content, and ChE activities. However, most of these correlations were driven by individual outliers. The only correlations that still showed clear trends after removal of outliers was a positive relationship between egg numbers produced and selenium concentrations and a negative correlation between blood butyryl ChE activity and lead concentrations. No pathologies as determined by liver

histology as a function of exposure to selected chemicals were observed. In summary, this study is of limited use for the UCR RI/FS for the following reasons:

- Limited utility of data due to exposure to a mixture of metals and organochlorine chemicals that differs from the scenario in the UCR.
- Limited interpretability of endpoints due to capturing stress as a likely confounder for the biochemical endpoints measured. This is especially true for the steroid hormone measurements that are known to change dramatically a short time after an organism has been stressed (e.g., Kubokawa et al 1999; Haddy and Pankhurst 1999).
- Unclear relevance of the effects. The only indicator of a biologically relevant response (liver histology) did not reveal any clear effects.

A5.1.6 Kruse and Scarneccia (2002b)

This study investigated the potential effects of various de-adhesion treatments commonly used during rearing of white sturgeon eggs on contaminant uptake and survival of embryos in Kootenay River water. Embryos were exposed to different matrices (unfiltered river water, filtered river water, river bottom sediment, suspended river solids, and Fullers Earth) and analyzed for selected metals, organochlorine pesticides, and PCBs. The exposure duration was from fertilization of eggs through 13 days post fertilization. The study found significant differences in embryo mortality among treatments groups, and it was concluded that two contaminants in the rearing medium, namely copper and Aroclor 1260, could have contributed to the decrease in survival of embryos. However, the authors also concluded that mortality rates in relation to contaminant exposure were not excessive, and that further studies would be necessary to be able to establish relationships between contaminant concentrations and survival of embryos. In summary, the data provided are of only limited utility in context with the assessment of COIs in the UCR for the following reasons:

- Only eggs from one single mating were used (i.e., no genetic diversity).
- Exposure was to a mixture of metals and organochlorines that differs from the scenario in the UCR.
- Mortality (only endpoint) could not be directly associated with exposure to contaminants due to confounders such as fungal growth and differences in type of solids/sediment.

A5.1.7 Dwyer et al. (2005)

Dwyer et al. (2005) reviewed the sensitivity of 20 threatened aquatic vertebrate species to a selection of contaminants (carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin) based on acute toxicity data. The COI of relevance to the UCR that was reviewed in this study was limited to copper. Among the different species assessed, three were paddlefish species: Atlantic sturgeon (*Acipenser oxyrhynchus*), shortnose sturgeon (*Acipenser brevirostrum*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*). Based on the analysis conducted by the authors, it can be concluded that sturgeon are among the more sensitive species when exposed to copper. However, the study also revealed relatively greater differences in the sensitivity among different sturgeon species with ranks between 1.5 and 10.5 out of 20 species (1 = most sensitive species; 20 = least sensitive species). Given these species-specific differences and the acute exposure in the data sets utilized by Dwyer et al. (2005), no direct conclusions for the assessment of the potential toxicity of COIs in the UCR to white sturgeon can be drawn from this work.

A5.1.8 USFWS and USGS (2008; Appendix A)

In 2007, the U.S. Fish & Wildlife Service (USFWS) and the U.S. Geological Survey (USGS) initiated a study to evaluate the acute toxicity of copper, chlorine, and three herbicides to two different life stages (30 and 160 days post swim-up) of white sturgeon from the Columbia River and the Kootenay River. The data indicated that earlier life stages (30 days post swim-up) were more sensitive to exposure with copper than older (160 days post swim-up) animals by approximately a factor of 50 (LC50: 4.9 vs. 249 µg/L in 30 vs. 160 day post swim-up fish, respectively). The authors reported a mean acute LC50 value of 4.9 µg/L, which is less than half of the EPA AWQC of 12 µg/L adjusted for hardness. It is unclear, however, at what specific life stage the USGS study was conducted, because swim-up typically describes a period of time that can last over a period of 1 week, and the authors did not provide any detailed information on how timing for this life stage was determined. The same is true for any other water quality parameters, with the exception of hardness, which represent important information for the evaluation of the validity of the approach. Without more details regarding the design of the studies, co-factors, and exact life stage (preferable as "days post hatch" or "days post fertilization"), it is impossible to assess the validity of the data provided here.

A5.2 2008 White Sturgeon ELS Toxicity Studies (ENTRIX, in prep.)

ENTRIX, in cooperation with the U of S, conducted a series of experiments in 2008 to assess the potential toxicity of COIs to ELS of white sturgeon. These experiments represented a multiple line-of-evidence approach that investigated the potential effects

of Columbia River water and selected COIs on hatchability and survival of early white sturgeon life stages in a subchronic setting. In addition, to facilitate extrapolation from controlled laboratory experiment data to assess effective concentrations of three key COIs in the Columbia River, namely copper, cadmium, and zinc, an acute 96-hour LC50 toxicity study was conducted to obtain Columbia River water specific water effects ratios (WERs)[2] for these three elements. This was done to assess the relative bioavailability of these metals in Columbia River water compared to laboratory water. A summary and interpretation of the preliminary data obtained during these studies is provided in the following sections.

A5.2.1 *In Situ* Assessment of Columbia River Surface Water in Canada

During summer 2008, experiments were conducted to assess the *in situ* toxicity of Columbia River water up- and downstream of Teck's Trail smelter to early white sturgeon life stages. The experiments were conducted in mobile laboratories (retrofitted trailers) in direct proximity to the river using a live-feed of river water. Sturgeon were exposed from 8 hours post fertilization through 60 dph. Study sites were the water intake for the City of Trail (upstream reference site) and the Waneta surface water sampling station just upstream of the U.S.-Canadian border (downstream site) (Figure A5-2). In addition, a clean water negative control consisting of filtered and dechlorinated tap water was tested in parallel to the riverine water experiment groups to control for possible upstream influences. River and control water were analyzed frequently (every 3 to 7 days) for concentrations of COIs and water quality parameters. A summary of test conditions is provided in Table A5-1.

Surface Water COI Concentrations and Water Quality

At the time this QAPP was written, data analysis had not been completed and results had not been validated, and as such, the information provided in this section should be viewed and interpreted cautiously and with the understanding that the information contained herein might be subject to change.

Preliminary data for selected COIs in surface water up- and downstream of Teck's Trail smelter and in the filtered city water control revealed statistically significant differences in concentrations of dissolved lead between the riverine sites (Figure A5-3). Median concentrations and upper 95th centiles during the course of the exposure studies were

[2]The water effects ratio (WER) is defined as the ratio of the toxicity of a metal in site water to the toxicity of the same metal in standard laboratory water. WERs may be used to derive site-specific limits for certain metals from national and state aquatic life criteria that were originally developed using laboratory toxicity data. The WER has been developed to compensate for site-specific biogeochemical factors such as hardness, alkalinity, organic carbon, etc., which can influence the bioavailability and toxicity of metals.

less when measured at Waneta compared to the upstream site at the city water intake station of the City of Trail. This is in accordance with the data for lead reported during the same period by Environment Canada in surface water at the sites Birchbank and Waneta, which are located up- and downstream of Teck's Trail smelter, respectively.[3] No statistically significant differences were observed among any of the treatment groups for copper and cadmium. With the exception of cadmium (upper 95th centile) concentrations observed during the course of the experiment were all below the chronic continuous criterion (CCC) for freshwater species adjusted to the average hardness of 72 mg $CaCO_3$/L observed during the course of the experiments at the riverine sampling sites (USEPA 2006b). However, there appeared to be a number of outliers for cadmium and lead that were defined as values greater than 10 times that measured at the same location before or after the sample under consideration was taken. Excluding these outliers would result in all measured concentrations being less than the CCC (Table A5-2). However, to ensure that these outliers did not represent a spike in waterborne concentrations of each element, samples that were defined outliers are currently being reanalyzed. Analysis and validation of data for other metals including zinc are still under way. There were no differences in dissolved organic carbon (DOC) and total organic carbon (TOC) among any of the treatment groups.

Embryo Mortality/Hatchability

There were no significant treatment differences in hatching rates between any of the treatment groups. Hatching started approximately 8 days post fertilization, and was completed on Day 12. Hatch rates ranged between 76 and 82 percent. Overall, there was slightly less hatching success in the riverine treatment groups, which was attributed to an increased number of fungal infections of eggs in these groups as assessed by visual observation (Figure A5-4). Hatching rate success was similar to those observed during other white sturgeon spawning experiments (personal communication with personnel from these institutions).

Fry/Juvenile Mortalities

There were no significant differences in mortalities among the different exposure groups (Figure A5-5). However, overall cumulative assessment revealed mortality rates that were heterogeneously distributed over the course of the experiment with the greatest number of fish dying between Day 23 and 34 after initiation of the experiment. This phase coincided with the transition of sturgeon to exogenous food. Similar "die offs" have been reported by other groups that routinely spawn and breed sturgeon such as

[3] http://waterquality.ec.gc.ca/WaterQualityWeb/dataDownload.aspx?stationId=BC08NE0005

the Kootenay Trout Hatchery, Canada, the Columbia Basin Hatchery, USA, and the University of California, USA (personal communication; see Figure A5-6). Based on the observations made by the majority of these institutions, it appears that the transition to exogenous feeding represents a sensitive period during the early development of white sturgeon, which is characterized by natural high mortality rates.

One additional factor that may have contributed to the increased mortality rates was the seeding density of fish per treatment chamber. While seeding rates were originally calculated such that they always were less than those recommended by the American Society for Testing and Materials (ASTM) (E1241-05) for fish in general, no current guidelines exist for the conduct of chronic white sturgeon ELS tests. The results of this study revealed a significant and strong linear relationship between seeding rates and number of dead fish (Figure A5-7). This linear model appears to be of general applicability because when applied to the seeding densities of sturgeon in the laboratory experiments (Section A5.2.2), it predicted mortalities in the controls precisely within an error margin of less than 5 percent. When stratifying by life stage, it could be shown that this relationship was unique to the transition of larva to exogenous feeding (Figure A5-7). This may be an indication that ELS of white sturgeon are particularly sensitive to competition during the transition to feeding. However, as soon as sturgeon successfully adapt to exogenous food, they seem to be more robust with regard to this factor as could be demonstrated by the lack of a relationship between seeding density and number of dead fish at this life stage (Figure A5-8). Limitation of food resources as a possible reason for the density-related increase in mortalities can be excluded because fish were fed *ad libidum*, and did not consume all food provided. Furthermore, there were no differences in water quality (dissolved oxygen, NH_3, nitrate, nitrite, pH, temperature, phosphate, DOC, TOC, etc.) between treatment chambers and groups, which could have explained elevated mortalities in the exposure systems with greater fish densities.

A5.2.2 Determination of Sensitivity of ELS of White Sturgeon to Cadmium, Copper, and Zinc

Toxicity of copper, cadmium, and zinc to ELS of white sturgeon was assessed using the same study setup as described for the "*In Situ* Assessment of Columbia River Surface Water in Canada" (Section A5.2.1). Sturgeon were exposed to five concentrations of each element under continuous flow-through conditions from 8–10 hours post fertilization through 60 dph (hatching occurred approximately 1 week after fertilization). Dissolved concentrations of metals and water quality parameters were analyzed frequently in each treatment group (every 3 to 7 days). A summary of test conditions is

provided in Table A5-3. Survival patterns in the controls were similar when compared to the *in situ* studies (Section A5.2.1) with slightly less overall mortality (68 vs. 80 percent in the lab water *in situ* controls). The lesser percent mortality can be explained by the lesser seeding density in the laboratory studies compared to the field controls. When a linear exposure-response function established for the *in situ* studies was applied to the original seeding densities (Section A5.2.1), it predicted accurately the observed mortalities (i.e., measured = 145 dead fish; predicted = 139 dead fish), indicating that mortality rates adjusted for original seeding densities were comparable between the field and the laboratory studies.

For all metals, there were concentration-dependent and statistically significant increases in mortalities at the greatest two or three concentrations tested (see Figure A5-9). One hundred percent mortalities occurred within the first 14 days of exposure post hatch at the greatest and two greatest exposure concentrations of cadmium and zinc, and copper, respectively. Increased mortalities also were observed for the 10.2 and 216 µg/L cadmium (100 percent mortality) and zinc (87 percent mortality) exposure groups, respectively. However, these could be attributed primarily to elevated mortality rates later during the experiments (juvenile stage). ELS of sturgeon were most sensitive to the exposure with cadmium and copper (Table A5-4). A summary of the test conditions is provided in Table A5-5. Based on the LC50s and lowest-observed-adverse- effects concentrations (LOAECs) for all elements and the NOAECs for cadmium, the national recommended water quality criteria for aquatic life (USEPA 2001, 2006b, 2007) would have been protective of this species.

A5.2.3 Determination of Columbia River-Specific Water Effects Ratios for White Sturgeon

To elucidate possible differences in the sensitivity of early white sturgeon to the same concentrations of COIs when tested in Columbia River water as compared to laboratory water, WERs were determined for cadmium, copper, and zinc in accordance with the methods described by USEPA (1984, 1994). These calculations were conducted to generate Columbia River-specific information for these three elements in support of the extrapolation of data obtained during the laboratory sensitivity studies (Section A5.2.2) to the situation in the Columbia River. WERs were calculated based on 96-hour LC50 studies with sturgeon life stages approximately 8–10 days post-hatch. This life stage was selected because it was assumed that sturgeon would be more sensitive during earlier stages, and because during this period, the fish are still in the yolk-sac stage, and thus, they did not need to be fed during the experiments. These experiments revealed that sturgeon were approximately 4 times less sensitive to exposure with cadmium and

zinc when exposed in Columbia River water but were slightly more sensitive (1.4-fold) when exposed to copper in river water (see Table A5-6 and Figure A5-10). The specific cause for these differences in sensitivity as a function of element is unclear, but it should be noted that dose-spacing was such for all elements that only 4-fold differences could have been detected statistically (doses increased in 4-fold increments). Furthermore, exposure concentrations presented here were nominal, and may have been different from absolute measured concentrations. Therefore, this information needs to be interpreted with care until the analytical data have been validated and are available. Analyses of test solutions for COIs are currently under way, and will be provided as soon as the data have been obtained and validated. Regardless of these uncertainties, it could be demonstrated that LC50s for white sturgeon after exposure to copper were in the same range as those reported by Dwyer et al (2005) for Atlantic and shortnose sturgeon, but were approximately 20 times greater than those reported by USFSW and USGS (2008). Furthermore, white sturgeon appeared to be approximately 2 and 20 times more sensitive to copper and cadmium than were Siberian sturgeon (Lapirova et al. 2000). However, the animals tested by Lapirova et al. (2000) were at a much later life stage, and thus, may have been less sensitive. Furthermore, there are a number of uncertainties related to the comparison of the data presented herein with the Dwyer et al. (2005) and the USFWS and USGS (2008) studies that are mainly due to lack of detailed information on the exact life stages tested (Dwyer et al. 2005) and test conditions under which both studies were conducted (including water quality and pre-conditioning of animals). The effect of size on sensitivity to copper, in particular, has been shown to be related to the turnover rate of internal sodium pools, which is inversely related to organism size (Grosell et al. 2002).

A5.2.4 Summary of 2008 White Sturgeon ELS Toxicity Studies

Based on the data obtained during the studies conducted in 2008 to assess the potential effect of COIs in Columbia River surface water up- and downstream of Teck's Trail smelter, there is no evidence that 1) surface waters at the investigated sites upstream of Teck's Trail smelter and downstream at Waneta were toxic to ELS of white sturgeon ranging from freshly fertilized eggs through 60 dph; and 2) COI concentrations present in the Columbia River at either of the investigated sites had the potential to cause increased mortality in the test population of white sturgeon at the life stages tested. Furthermore, comparisons of concentrations of the three COIs investigated during the 2008 laboratory studies revealed that maximum concentrations observed for cadmium, copper, and zinc during the past 8 years at Waneta were less than the NOAECs (Table A5-4 and http://waterquality.ec.gc.ca/WaterQualityWeb/dataDownload.aspx).

A6 DATA GAPS

A series of studies has been conducted to assess the potential effect of COIs on ELS of white sturgeon both under controlled laboratory conditions and in the field (Section A5). While some of this information is helpful in assessing the relative sensitivity of ELS of white sturgeon to select COIs, as well as potential issues related to the exposure situation in the Columbia River upstream of the U.S.-Canadian border, the following data gaps and resulting uncertainties still remain.

A6.1 Potential Surface Water Toxicity Downstream of the U.S.-Canadian Border

While data obtained during studies conducted during 2008 in the Canadian section of the river up- and downstream of Teck's Trail smelter indicate that there is no apparent direct toxicity to ELS of white sturgeon including fertilized eggs/embryos, fry, and juveniles, it remains unclear whether there are potential effects of water-borne COIs in the Site.

There are limited data available on the exact location of the nursing grounds of white sturgeon within the reach of the Columbia River between Grand Coulee Dam and the Hugh L. Keenlyside Dam. However, it has been hypothesized, that the river in the vicinity of Marcus Flats represents a likely habitat for sturgeon fry and juveniles from adults that spawned at Waneta (Golder 2007), and which is supported by a study that found greatest white sturgeon larvae abundances between Northport and upstream of the area of Marcus Flats (Howell and McLellan 2006). Analyzing the potential effects of COIs in these areas is of particular relevance as such investigations integrate the numerous co-factors unique to this habitat that can affect the *in situ* toxicity of chemicals. In this particular case, such factors would include:

- Potential transfer of COIs from pore water of granulated slag/sediment deposits to the overlying surface water
- Potential increase concentrations and/or alteration of patterns of COIs due to upstream sources (e.g., Pend Oreille River, historical Le Roi smelter at Northport)
- Potential differences in water quality conditions (e.g., dissolved oxygen, ammonia)
- Altered toxicity of COIs, specifically metals, due to presence of site-specific ligands.

Thus, to enable the risk assessment of near-bottom water exposure in the UCR in support of the RI/FS, toxicological information specific to this matrix should to be collected.

A6.1.1 Life-Stage Specific Sensitivity of White Sturgeon

Information provided by USFWS and USGS (2008) (see Section A5.1.8) suggested that white sturgeon may have certain windows during their early development when they might be unusually sensitive to chemical (or metal) exposure. However, there is only limited information available from which to address this hypothesis, and that is for a single COI, copper (see also discussions in Section A5.2.2). Therefore, there is a need to address the question of whether certain life stages of white sturgeon are more sensitive to exposure with COIs, and if so, what the relevance of this would be for risk to fish in the UCR.

A6.2 Relative Sensitivity of ELS of White Sturgeon in River Water to Lead and Copper

During the 2008 sturgeon ELS studies, the sensitivity of white sturgeon to three COIs—copper, cadmium, and zinc—was assessed. However, there are still some uncertainties remaining with regard to the possible effects of other COIs, such as lead. In response to the question about whether lead in the UCR may pose a toxicological risk to ELS of sturgeon, studies are proposed to assess the relative sensitivity of this species to lead both under laboratory conditions and in river water. Furthermore, given the uncertainties related to the WER determined for copper during the 2008 studies, refinement of experiments is required to help address remaining questions, including re-defining dosing ranges with less spacing between test concentrations.

A7 DATA QUALITY OBJECTIVES, CRITERIA, AND DESIGN RATIONALE

EPA's seven-step DQO process (USEPA 2006a) was used to guide the design rationale for the 2009 sturgeon ELS studies. The DQO process is a tool to determine the type, quantity, and quality of data that are needed to address specified risk questions. This process establishes performance and acceptance criteria for the data to promote achievement of study goals. Briefly, these DQOs are (USEPA 2006a):

DQO Step 1: State the problem—This step describes the problem and clearly states the questions that will be addressed by the data being collected. In doing so, it identifies the type(s) of data that will be needed, the planning team, and the proposed schedule.

DQO Step 2: Identify the goals of the study—The second step identifies the goals and desired outcomes of the study and how the information will be used in the decision-making process. Furthermore, this step states the null hypotheses that will be tested by the proposed studies.

DQO Step 3: Identify information inputs—This step determines the types and sources of information that are needed to resolve the decision statement.

DQO Step 4: Define the boundaries of the study—This step specifies the spatial and temporal features pertinent for decision making. Any practical constraints that could interfere with sampling also are identified in this step.

DQO Step 5: Develop the analytical approach—This step defines the parameters of interest, specifies the type of inference, and develops the logic for drawing conclusions from the data.

DQO Step 6: Specify performance and acceptance criteria—This step derives the performance or acceptance criteria that the collected data will need to achieve. This is in addition to specifying the appropriate level of laboratory quality assurance practices and guides the study design for new data collection or procedures to acquire and evaluate existing data relative to the intended use.

DQO Step 7: Develop the study design—The final step is development of a resource-effective design for collecting the proposed (environmental) samples in a manner that that will achieve the specified performance criteria. This will be followed by development of a study-specific QAPP.

A7.1 Step 1—State the Problem

This section describes why there is a special study of white sturgeon (*Acipenser transmontanus*) in the UCR RI/FS, a species whose sensitivity to chemicals is poorly understood. An extensive review of the aquatic toxicological literature concerning what is known about white sturgeon in the UCR and the sensitivity of sturgeon relative to other fish and aquatic invertebrates are presented.

White sturgeon is a species of special interest in the UCR ecological risk assessment because of its poor recruitment in the Site over the past decades, because it may be sensitive to metals, and because its epibenthic habitat and benthic feeding potentially increases the likelihood of exposure to metals through contact with sediments or bioaccumulation in its benthic prey. For example, elevated metal residues have been measured in species that live in Lake Roosevelt and have similar habitat preferences and

food habits, namely largescale sucker (*Catostomus macrocheilus*) (Johnson and Serdar 1991; USEPA 2005b), suggesting white sturgeon may experience elevated exposures to sediment metals.

Poor recruitment of white sturgeon in the Columbia River between Grand Coulee Dam and Hugh L. Keenlyside Dam (Lower Arrow Lake, B.C.) in Canada has been documented since the 1970s. While both spawning of adults and occurrence of embryos and larvae have been reported frequently during the past years, limited embryos and larvae have been captured in nets set from the Canadian border to Evans, Washington (Howell and McLellan 2006). Furthermore, juveniles (9–10 months old) that have been released into the Canadian section of the Columbia River as part of the White Sturgeon Recovery Plan appear to have good survival, growth rates, and body condition—Howell and McLellan 2006). Therefore, survival of one or more stages during the early development of larval and juvenile sturgeon appears to be limiting recovery of the population (Howell and McLellan 2006).

Several potential causes have been suggested for the low survival of young of the year sturgeon. They could be acting singly or in some combination, and they include limited habitat; flow regimes; contaminants in the water, sediments, and food; food supply; inbreeding; predation by introduced species such as walleye and smallmouth bass; competition with other fish species; and parasites and pathogens (UCWSRI 2002; Kruse and Webb 2006). This study seeks to define the role of contaminants in near-bottom water to determine if it is a limiting factor on the survival, growth, and development of larval and juvenile sturgeon. As previously noted, there are three possible exposure pathways—water, sediment, and diet. This QAPP focuses only on the water pathway. The other two pathways (sediment and diet) may be considered for further evaluation at a later date.

Overall, toxicity data for several species that live on or near the bottom in the UCR (i.e., white sturgeon, mountain whitefish, and largescale sucker) are extremely limited for chemicals in general, let alone all the COIs identified for the UCR site. Furthermore, the limited toxicity data available for these species and closely related surrogate species are based on acute rather than subchronic or chronic exposures. The acute data suffice to index relative sensitivities to acute exposures as well as chronic toxicity, provided the chemical's acute and chronic modes of action are the same. But where the acute and chronic modes of action differ, acute toxicity data cannot be extrapolated to estimate chronic toxicity without chronic toxicity tests.

A7.1.1 Team Members and Roles

One of the goals of Step 1 of the DQO process is to establish a planning team and identify decision makers. The planning team will consist of personnel from ENTRIX and U of S, and will be supported by the Teck technical team. A detailed overview of the specific personnel and their roles is provided in Section A4.2.

A7.1.2 Resources and Timelines

To conduct the experiments identified in this QAPP, toxicity testing will be conducted at field laboratories situated at one site in the vicinity of Marcus Flats just upstream of Kettle Falls in the UCR, one site at Waneta directly upstream of the U.S.-Canadian border, and at one site situated at a reference location upstream of Teck's Trail smelter. Details are provided in Section B1.

Timing of aquatic toxicity studies with ELS of white sturgeon will be dictated by the spawning season of white sturgeon in the Columbia River, and which is expected to take place sometime between June and July. Thus, this period represents the only window when embryos and juveniles are available for testing. As stated in Section A4.1, herein we refer only to tests that are scheduled for 2009.

In situ studies to be conducted in 2009 using field laboratories in direct proximity of the river are as follows:

- 60+ day, subchronic toxicity testing to sturgeon embryos and juveniles of water near to the bottom at a UCR site in the lower Marcus Flats in the U.S. and at two sites north of the U.S.-Canadian border
- Determination of the WER based on acute LC50 studies for periods of 6 to 96 hours for aqueous exposures of an ELS of white sturgeon to lead and copper (8–10 dph)
- Validation study using copper as the reference toxicant that will be conducted parallel to studies at the USGS Columbia Environmental Research Laboratory: two 96-hour LC50s for exposure of ELS of white sturgeon at Days 8 and 40 post hatch to copper.

A7.2 Step 2—Identify the Goal of the Study

The goal of this study is to determine if there are unacceptable risks to early life-stages of white sturgeon from exposure to COIs in UCR near-bottom water. These studies will be the first step in a multiple lines-of-evidence approach of the assessment of the potential toxicity of COIs in UCR matrices to ELS of white sturgeon (Figure A7-1).

Specific risk-related questions that will be addressed during the 2009 sturgeon ELS studies are:

- What are the concentrations of COIs in near-bottom water in and upstream of the Site during the reproductive season of adult white sturgeon and during the time when early white sturgeon life-stages are present?
- What are the acute and/or subchronic effects on survival, growth, and development of ELS of white sturgeon from exposure to UCR near-bottom water?
- What is the bioavailability to ELS of white sturgeon of cadmium, copper, lead, and zinc in near-bottom waters using 1) site-specific WERs (USEPA 1994; Paquin et al. 2000), and 2) the biotic ligand model (Di Toro et al. 2001, 2005).
- Are there any differences in acute and/or subchronic effects on survival, growth, and development of ELS of white sturgeon between the upstream and downstream study sites? If significant differences occur:
  - What is the magnitude of these effects, and could they result/predict unacceptable risks to the UCR white sturgeon population
  - Are these effects correlated in any way to elevated COI concentrations at the downstream sites?
- What are the acute concentration-response relationships from exposure of ELS of white sturgeon to aqueous copper at ~8 and ~40 dph.
  - How do these relate to concentrations of copper measured in the UCR in the water column and in the water near the river bottom considering acute to chronic ratios for copper?
- What is the Columbia River-specific WER for lead and copper (to be used as an adjustment factor accounting for the effect of site-specific water characteristics)?

A7.2.1 Testable Null Hypotheses

1. There are no statistically significant differences in hatchability, survival, and development of ELS of white sturgeon between the lab-water controls and the site upstream of Teck's Trail facility.
2. There are no statistically significant differences in hatchability, survival, and development of ELS of white sturgeon exposed to near-bottom water between the up- and downstream study sites.

3. There are no statistically significant differences in hatchability, survival, and development of ELS of white sturgeon between the two field sites downstream of Teck's Trail facility.
4. There are no statistically significant differences in the sensitivity of different ELS of white sturgeon to selected COIs.

A7.3 Step 3—Identify Information Inputs

Step 3 of the DQO process (USEPA 2006a) requires consideration of the following:

- The types and potential sources of information (e.g., site characteristics or variables) that should be measured to provide estimates or resolve decisions.
- Information to provide a basis for specifying performance or acceptance criteria.
- Information on the performance of appropriate sampling and analytical methods.

The decision process regarding the white sturgeon toxicity assessment will be supported by the following measurements:

1. Exposure and water quality data
    a. Metals and metalloids in the total and dissolved fractions of water samples, namely: Standard and selected other metals listed in the target analyte list (i.e., beryllium, boron, aluminum, titanium, vanadium, chromium, manganese, iron, copper, nickel, copper, zinc, arsenic, selenium, strontium, molybdenum, silver, cadmium, tin, antimony, barium, mercury, thallium, lead, uranium)
    b. Conventional water quality parameters and those required to assess and evaluate bioavailability (http://www.hydroqual.com/wr_blm.html):
        i. Alkalinity, pH, dissolved oxygen, DOC, TOC, hardness, major cations (Ca, Mg, Na and K), major anions (SO_4 and Cl), sulfide, total dissolved solids, total suspended solids, and temperature, (HydroQual 2005)
        ii. Other potential toxicants (ammonia, manganese)
        iii. Nutrients: nitrate, nitrite, total phosphorus
2. Determination of biological endpoints in ELS of white sturgeon
    a. Semichronic *in situ* river water exposure studies

i. Hatchability: NOAEC/LOAEC

ii. Fry/juvenile mortality: NOAECs and LOAECs

iii. Growth (length and mass): NOAECs and LOAECs

iv. Developmental abnormalities for defined exposure durations

b. Laboratory toxicity testing of metals

i. Mortality: NOAECs, LOAECs, and LC50s after 6, 12, 24, 48, 72, and 96 hours of exposure

3. Determination of toxicological benchmarks from the literature, with primary use of EPA acute and chronic ambient water quality criteria when available (http://www.epa.gov/waterscience/criteria/wqctable/index.html).

A7.4 Step 4—Define the Boundaries of the Study

This step specifies the population of interest for the study, the geographical boundaries of the site, and any temporal considerations that may be required.

A7.4.1 Target Populations for Risk Evaluation

The study site encompasses the Columbia River between Grand Coulee Dam and the U.S.-Canadian border because white sturgeon occur throughout; however, the study will focus on the UCR from Marcus Flats upstream, an area where white sturgeon larvae have been reported at greatest abundances compared to further up- and downstream sites (Howell and Howell 2006).

Target populations of interest for risk evaluation are the white sturgeon that live in the UCR and north of the U.S.-Canadian border:

- Represented by locations in the vicinity of Marcus flats and Waneta in the *in situ* studies.
- UCR-wide assessment/prediction by COI-specific data obtained during controlled laboratory studies.

A7.4.2 Geographic Boundary of the Site

The Site encompasses the UCR from the U.S.-Canadian border (River Mile [RM] 745) to the Grand Coulee Dam (approximately RM 596). The Site has been divided into six reaches as previously identified in the draft RI/FS work plan and draft SLERA, of which Reaches 1 through 3 will be the focus of the 2009 white sturgeon toxicity studies.

- Reach 1 (U.S.-Canadian Border at RM 745 to RM 730)—riverine
- Reach 2 (RM 730 to RM 712)—transitional (riverine to lacustrine)
- Reach 3 (RM 712 to RM 700)—Marcus Flats [transitional (riverine to lacustrine)]

A7.4.3 Temporal Considerations

The temporal boundaries of the studies are defined by the reproductive cycle of white sturgeon. Typically, spawning of white sturgeon in the UCR occurs sometime in June or July depending on temperature and hydraulic conditions. Thus, onsite study preparations would commence in late April/early May so that exposures can be initiated as soon as fertilized sturgeon eggs are available in June/July.

A7.5 Step 5—Develop the Analytical Approach

The risks of the exposure of ELS of white sturgeon to UCR near-bottom water will be assessed in a deterministic fashion using a multiple lines-of-evidence approach: 1) *in situ* toxicity testing of river water, and 2) comparison of *in situ* metal concentrations to 2a) subchronic NOAECs for white sturgeon, and 2b) water quality criteria.

The first line of evidence will be derived from *in situ* acute and subchronic toxicity testing of near-bottom water. Long-term (> 60 days overall exposure time), continuous-flow exposures of white sturgeon larvae, fry, and juveniles to ambient waters will be tested by pumping near-bottom water into test chambers of a field laboratory. The tests will begin with newly fertilized eggs and continue to 60 dph. Effects on survival, growth, and development (gross internal and external morphology) will be measured. Complete details on the experimental design are provided in Section B1.4. If the *in situ* testing reveals significant toxicity in river water vs. the lab water control, or between the up- and downstream sites and/or the lab water control, then the first question is whether the results can be explained by *in situ* water chemistry. If the answer is yes, results for the three lines of evidence are used in the BERA. If not, then no further investigation of the cause(s) of toxicity is conducted in support of the RI/FS.

The certainty of the responses cannot be determined *a priori*. However, decision rules can be specified. Decision rules will be dependent on the presence of a relative response, the statistical vigor of that response, and the correlation of exposure to the response (i.e., dose response curve). Decisions rules for dose will be based in a large part on the usability of the analytical data for the exposure calculation.

Depending on the various outcomes of those results, a decision will be made whether the data are sufficient to conclude whether further evaluation and/or studies are necessary and/or whether a technically valid conclusion can be made.

A7.6 Step 6—Specify Performance and Acceptance Criteria

Specifying limits on decision errors involves defining the possible decision errors and the consequences of making these errors. Typically, this is done by describing the decisions in terms of hypothesis tests or other objective decision criteria and by specifying the hypotheses to be tested using an appropriate statistical model. Limits can also be specified by identifying the decision errors as false-positive and false-negative errors. In this study, the type I error (the false positive decision error; α) will be set at 0.05. The type II error (the false negative decision error; β) will be set at 0.2.

Testing will meet the quality criteria and quality assurance criteria defined in the following documents:

- ASTM. 2005. Standard guide for conducting early life-stage toxicity tests with fishes. ASTM E 1241 – 05. American Society for Testing and Materials, West Conshohocken, PA, USA
- ASTM. 2007. Standard guide for conducting acute toxicity tests on test materials with fishes, Macroinvertebrates, and Amphibians. ASTM E729 – 96. Developed by Subcommittee: E47.01 |Book of Standards Volume: 11.06. Philadelphia, PA, American Society for Testing and Materials: 22 pp.
- ASTM. 2008. Standard guide for conducting acute toxicity tests on aqueous ambient samples and effluents with fishes, macroinvertebrates, and amphibians. ASTM E1192 – 97. American Society for Testing and Materials, West Conshohocken, PA, USA.
- USEPA. 1996. Sampling ambient water for trace metals at EPA water quality criteria levels. EPA-821-R-96-011. U.S. Environmental Protection Agency, Office of Water, Washington, DC.
- USEPA. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5th Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC, 275.
- USEPA. 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Details on performance criteria are provided in Section B1.4

A7.6.1 Optimized Study Design

Details concerning how data will be collected for assessing the potential toxicity of COIs present in near-bottom water to white sturgeon are provided in the SOPs associated with this QAPP. These methods will be tailored to the physical and logistical constraints associated with obtaining the most effective endpoint measurements. The remainder of this section is intended to describe the methods that will be used to determine sample sizes needed to meet the objectives of the study. The method chosen will ultimately depend on the stated end use of data generated for each line of evidence, the availability of relevant and sufficient historical information on variation in the measurement endpoints, the selection of type I (false-positive; α) and type II (false-negative; β) error values for relevant magnitudes of differences between test group means. The method that will be used to calculate statistical power of the studies to be conducted under this project is the hypothesis testing method (see below). This method uses estimates of variance (S^2) to determine the optimal sample size (n). One of the key parameters in determining the required sample size is the relative difference to be demonstrated. The relative difference (relative to the mean) is the property that affects sample size. To demonstrate differences with the same power, a larger sample size would be required to demonstrate a relative difference of 5 percent of the mean than to demonstrate a 20 percent difference. Furthermore, the ability to demonstrate a particular difference relative to the mean is dependent on the variance of the population. As an example, a greater sample size would be required to demonstrate difference between two means when the coefficient of variation is 40 percent than when it is 20 percent.

Statistical Power for Hypothesis Testing of Differences among Treatment Means

Statistical power of the proposed study designs was calculated using the "Means Routine" of the PASS software of NTCC (NTCC, Kaysville, UT, USA). This method allows the specification of the level of type II error (β). This method determines the required number of samples (replicates of the basic experimental unit, which in this study is the replicate exposure system) necessary to achieve adequate statistical power in testing the null hypothesis, H_0, using a fixed significance level α (i.e., the type I, or false-positive error) and a fixed, specific alternative hypothesis, H_1. This fixed alternative may be for hypothesis tests involving a single mean (e.g., comparing the mean number of mortalities at one site to COI concentrations) or for problems involving two means (e.g., comparing the mean mortality at different sites). The key to this method is determining the relative magnitude of difference to be demonstrated, relative

to the variance of the mean or means. Thus, it is the relative magnitude that is important, not the absolute difference between means. To effectively use this method, in addition to selecting values for the probability of committing type I and type II errors, the method requires estimates of the variance of each population as well as the magnitude of difference to be demonstrated for a particular mean value.

Based on the above method and the information collected during the 2008 surface water toxicity studies (means and variance) the number of replicate groups required to detect significant differences among sites for αvalues of 0.05 and 0.1 and β values of 0.2 and 0.1 were calculated (see Table A7-1). Based on these calculations, three or more replicate groups provide sufficient power to detect 10 percent or greater increases in overall mortality among sites. Given the logistical restraints of the studies proposed herein it was decided to used four true replicate groups, which should permit detection of a 7 percent increase in mortality with a power of greater than 80 percent and an α of 0.05.

A7.7 Step 7—Develop the Study Plan

Detailed discussions of the various study components are presented in Section B1 of this document.

A8 SPECIAL TRAINING/CERTIFICATES

The project manager is responsible for assembling a project team with the necessary experience and technical skills. Part of the process is to identify special training requirements or certifications necessary to execute the project successfully. Project-specific requirements include training specific to the analytical methods to be conducted, handling, and health assessment methods for white sturgeon larvae, usage of the flow-through exposure systems, and health and safety training for personnel engaged in onsite and laboratory activities.

All project personnel will receive training before commencing work onsite to ensure they are familiar with the required SOPs and safety and emergency procedures and are adequately skilled at collecting data and operating and maintaining the exposure system. Personnel training records are maintained by the laboratory manager.

In addition, all personnel working at the site should have the appropriate health and safety training identified in the health and safety plan (Appendix B).

A9 DOCUMENTATION AND RECORDS

This section identifies onsite and laboratory (analytical and U of S) records to be maintained for this project, information to be included in project reports, the data reporting format for data report packages, and the document control procedures to be used.

A9.1 Required Records

The critical records required for this project are identified below with descriptive or supporting information as appropriate. The records will include:

- Exposure system maintenance logs and sample collection records including notebooks, photographs, and any other records used to record raw data. General procedures will be referenced in the experimental notes, while any necessary deviations or modifications required to operate the exposure systems or to collect samples will be described in detail
- COC records
- Corrective action reports
- Sample ID, treatment, matrix and dilution factor (whatever applicable)
- Sample receipt and analysis dates
- Result/assessment and re-analyses (if necessary)
- Final analyte concentration including reporting limit, laboratory qualifiers, and reanalyses
- Percent recovery of each compound in the matrix spike sample
- Matrix spike recovery control limits;
- Relative percent difference (RPD) for all matrix spike/matrix spike duplicate (MS/MSD) and/or laboratory control sample (LCS)/LCS duplicate (LCSD) results
- RPD control limits for MS/MSD and/or LCS/LCSD reports
- Laboratory control sample results when analyzed
- Recovery control limits for LCS or standard reference material recoveries and relative standard deviation
- Blank results for method blanks, experimental blanks, and equipment blanks
- Method blank summary indicating associated samples

- Case narrative.

For data validation, the following additional data will be required:

- Sample receipt/sample log-in forms
- Calibration information, including initial calibration, concentration response data of the calibration check standards, continuing calibration check data, instrument tunes, and associated samples

All raw data and logs will include the following information:

- Analyst's initials and date
- Initial and final sample and extract volumes or weights and/or dilutions
- Condition of instrument
- Documentation linking sample analysis to instrument calibration (where appropriate)
- Time of start of analysis of all experimental and quality control samples
- Instrument run log showing analytical sequence
- Dilutions performed and amount of sample analyzed
- Experimental samples, quality control samples, and blanks clearly labeled
- Quantification reports
- Sample preservation (where applicable).

Paper copies of all of these records will be retained. In addition, the laboratory will provide 1) an electronic deliverables in an ASCII comma-delimited format for all test results, and 2) an electronic backup for all onsite and laboratory data generated.

Procedures for project control, archiving, and storage of laboratory records are described in Section B10 of this QAPP. ENTRIX and U of S will adhere to a record retention time of 10 years for all laboratory records for the project.

A9.2 Project Reports

Several types of reports will be produced during the course of this project. The project manager will prepare summary reports for investigations described herein. Furthermore, data obtained during the studies described herein will be submitted in

form of technical report publications and manuscripts in the peer-reviewed scientific literature, including summary reports of data and quality assurance determinations.

A9.3 Record Maintenance and Storage

All documents relating to the project will be controlled to ensure proper distribution, filing, and retrieval, and to ensure that revisions are properly recorded, distributed, and filed.

Project records will be stored and maintained by ENTRIX. The project manager and office staff are responsible for organizing, storing, and cataloging all project information and for collecting records and supporting data from project team members. Once project records are cataloged, ENTRIX will ensure that the project records are appropriately filed by category in the correct project file. Filed documents are available to U of S and ENTRIX staff through checkout procedures developed to ensure the integrity of the project file. Individual project team members may maintain separate files or notebooks for individual tasks. These files or notebooks are transferred to the project manager as part of project closeout. The archived files will be stored and maintained by ENTRIX. Newly created documents will be transmitted to Teck quarterly in accordance with its document retention policy. Additional information on record management can be found in Section B10 of this QAPP.

SECTION B: DATA GENERATION AND ACQUISITION

This section describes all aspects of measurement, design, and implementation and discusses the methods that will be used for sampling, analysis, data handling, and quality control in support of the studies that will be conducted in 2009 to assess the potential toxicity of UCR near-bottom water to ELS of white sturgeon. These studies will include the following experiments:

- Chronic *in situ* studies with river water at two locations in the Canadian reach and one location in the U.S. reach of the Columbia River between Hugh Keenlyside and Grand Coulee dam
- Acute toxicity study to determine the WER of lead and copper between Columbia River water and control water
- Acute ELS toxicity studies in support of an intra-laboratory validation experiment.

The approach is designed to collect data that supports characterizing the nature and extent of potential near-bottom water-related toxicity to ELS of white sturgeon from fertilized eggs through 60 dph. It will provide detailed information for collecting the proposed samples in a manner that that will achieve the specified performance criteria. The study approach was developed based on information from previous investigations (Section A5.2), the preliminary CSM; information available on the ecology of white sturgeon in the UCR; and available exposure data of sediments and water (Howell and McLellan 2006; Paulson and Cox 2007; Besser et al. 2008).

The following specific aspects of measurement and data acquisition will be covered in this section:

- Sampling process design and rationale
- Sampling methods requirements
- Sample handling and custody requirements
- Analytical method requirements
- Quality control requirements
- Instrument/equipment testing, inspection, and maintenance requirements
- Instrument calibration and frequency
- Inspection and acceptance requirements for supplies and consumables

- Data acquisition requirements
- Data management.

B1 SAMPLING PROCESS DESIGN AND RATIONALE

B1.1 Test Species—Numbers, Source, Strain, and Life-Stages

Species: White sturgeon (*Acipenser transmontanus*)

Strain: Offspring from wild sturgeon caught in the Columbia River at Waneta

Age: Eggs, larvae, and juveniles

Number: 40,000 eggs from 2 to 4 breeding pairs

Source: Kootenay Trout Hatchery, Fort Steele, B.C., Canada

Freshly fertilized white sturgeon eggs from at least two breeding pairs from the Columbia River at Waneta will be obtained from the Kootenay Trout Hatchery, Fort Steele, B.C., Canada. Fertilization of eggs will be harmonized in the hatchery by injecting adult riverine sturgeon with a gonadotropin analog on two subsequent days. Eggs will be transported to the exposure facilities between 4 and 8 hours after fertilization. Acute WER and life-stage sensitivity studies will be conducted using fry/juveniles hatched from the same batch of eggs. These animals will be held under control conditions until initiation of experiments. Arrangements with the Kootenay trout hatchery will be made to retain a contingent of fish from the same fertilization event at the hatchery (~3,000 fry) as a backup if mortality rates in the controls are too great to be able to reinitiate studies at a later life stage.

B1.2 Study Locations and Rationale

A total of three locations shall be investigated within the above defined site (Figure B1-1). One location is located on a property owned by the City of Trail just upstream of Teck's Trail smelter (49°07′01.32″N; 117°43′27.25″W). This location will serve as a riverine reference site with the aim to control for any upstream influences. The second location upstream of the U.S.-Canadian border is located at the Waneta surface water sampling station (49°00′28.35″N; 117°36′56.69″W) and reflects an exposure situation primarily influence by Teck's Trail facility. The third location will be just upstream of Kettle Falls in the lower area of Marcus Flats (48°38′02.54″N; 118°07′06.09″), which is anticipated to represent an important nursing ground for sturgeon and has also been identified as

containing deposits of granulated slag. Selection of the study sites was based on two main factors: exposure regime and feasibility. The rationale for the choice of location was as follows:

1. Reference site: To enable the assessment of potential toxicities in surface water associated with releases by Teck's Trail smelter, it is necessary to compare findings from the potential exposure sites to a reference within the same system that is not nor has been exposed to any materials released by the smelter. Such a reference location allows controlling for potential other factors in river water that may affect early sturgeon development and/or survival. Furthermore, water from this site will be used to develop WERs for lead and copper. The reference site is located at the city water intake of the City of Trail on the northeast shore of the Columbia River opposite and upstream of Teck's smelter facility (49°07'01.32"N; 117°43'27.25"W). A working relationship between ENTRIX, the U of S, and the City of Trail was established in 2008, and the site has been set up to provide electricity and water for the studies.

2. Canadian downstream site at Waneta: This location is situated at the river just north of the U.S. border and the confluence with the Pend Oreille River at the Waneta surface water sampling station (49°00'28.35"N; 117°36'56.69"W). The river at Waneta Eddy just downstream of this location represents an important spawning ground for white sturgeon. This allows the assessment of potential surface water toxicity at this biologically significant site to sturgeon using offspring of sturgeon that would have spawned in this area. Finally, logistics for conducting flow-through *in situ* studies with river water at this site were already established in 2008.

3. U.S. location at Marcus Flats: The decision was made to select a study location characterized by a situation where the water would have been flowing over large stretches of slag containing sediments. Based on the information available on slag deposits in the river (e.g. NHC 2006) and hydrological conditions, a site was selected that is located a short distance upstream of the old Kettle Falls in lower section of Marcus Flats (48°38'02.54"N; 118°07'06.09").

B1.3 Support Facilities for Sampling Methods

The primary laboratories for analysis of biological samples collected for this study will be:

- Environmental Toxicology Group, U of S, Saskatoon, SK S7N 5B3, Canada.

The primary laboratories for analysis of samples for COIs will be:

- Columbia Analytical Services, Longview, WA, USA.

B1.4 Experimental Setup and Sampling Strategy

B1.4.1 *In Situ* Surface Water Toxicity Studies

Exposures will begin immediately after transfer to the exposure sites and will continue through approximately Day 60 post hatch (estimated pre-hatch exposure time is 6–7 days, resulting in an approximately 66-day total exposure time). Larvae from the different breeding pairs will be combined and randomly assigned to treatment containers. Subsamples of sturgeon will be taken from all treatments at different times during the 66-day exposure period that will be in accordance with key developmental stages (Figure B1-2).

In situ exposure of sturgeon eggs and larvae will be conducted in direct proximity to the Columbia River using an acclimatized modular structure that allows controlling light and temperature. The study will utilize river water (upstream of the Trail facility) as an environmental reference in addition to a filtered city water (field lab water) control. Each treatment group and the controls/reference will be tested in four replicate exposure systems each featuring three replicate streams (Figure B1-3). Each exposure system is temperature controlled using individual chilling units. Water intakes in the river will be as close to the trans-boundary water layer between sediment and near-bottom water as possible without the risk of aspirating particulates from the sediment layer. Non-metal well-heads will be used to pump water from bottom at the sites where no pre-installed water intakes are available (e.g. at the Marcus Flats site).

Sampling design including fish densities, time of exposure initiation, replicate treatments, etc., was based on the experiences gained during the 2008 surface water toxicity studies conducted by ENTRIX and the U of S (in prep.). All criteria exceeded requirements listed in ASTM guidelines for ELS testing of fish (Table B1-1; ASTM 2005). General conditions for husbandry of ELS of white sturgeon were adapted in accordance with hatchery protocols and after discussion with hatchery personnel where appropriate. Summaries of test conditions for conducting the studies, the general activity schedule, and the test acceptability requirements are provided in Tables B1-2 through B1-4, respectively. Fish densities for specific life-stages during the exposure period were calculated based on the linear regression model provided in Section A5.2.1 (Figure A5-7).

B1.4.2 Determination of the Water Effects Ratio for Lead and Copper at the Upstream Field Site Upstream of Trail, B.C., Canada

WERs in support of the field- and laboratory based assessment of the toxicity of lead and copper to ELS of white sturgeon will be conducted as described by USEPA (1984, 1994) and in accordance with requirements listed in ASTM guidelines for ELS testing of fish (ASTM 2005) and conditions determined during the 2008 white sturgeon ELS toxicity studies (ENTRIX, unpublished data) using a static renewal exposure system. Acute (96-hour) LC50 values will be determined both in river water obtained from the Columbia River at the upstream field site (city water intake of the City of Trail, B.C., Canada) and in control laboratory water. Prior to initiation of the studies, fry will be acclimated to the incubation cups for 24 hours. Lab water hardness, pH, and temperature will be adjusted such that they approximate conditions in Columbia River reference water (upstream site). Concentrations for lead were selected based on concentrations reported to be toxic to trout (Mebane et al. 2008). All concentration increments follow a 3-fold geometric series. Concentration will be adjusted for river water background levels of COIs so that final concentrations of lead are directly comparable between lab- and reference-water exposures. Concentrations for copper were selected based on the preliminary WER studies conducted with this metal during the 2008 surface water white sturgeon toxicity studies (ENTRIX, in prep.). WERs will be calculated using the formula described below:

$$WER_{ColumbiaRiver} = \frac{LC_{50(lab\text{-}control)}}{LC_{50(field\text{-}reference)}} \qquad \text{(Eq. B1-1)}$$

Lead and copper concentrations to be tested will be 0, 1, 3, 9, 27, 81 and 243 µg/L, and 0, 1, 3, 9, 27, 54 and 108 µg/L, respectively. Summaries of test conditions for conducting the studies, general activity schedule, and test acceptability requirements are provided in Tables B1-5 through B1-7, respectively.

B1.4.3 Assessment of Life-Stage Specific Sensitivity to Copper

To assess the potential life-stage-specific sensitivity of white sturgeon to metals, a series of 96-hour LC50 studies in accordance with requirements listed in ASTM guidelines for ELS testing of fish (ASTM 2005) and conditions determined during the 2008 white sturgeon ELS toxicity studies (ENTRIX, unpublished data) will be conducted. Specifically, the acute toxicity of copper will be assessed at Days 8 and 40 post hatch in coordination with a parallel study by the USFWS laboratory in Columbus, Missouri. All laboratories will use fish from the same batch of eggs to be obtained from the Kootenay Trout Hatchery. Concentrations to be tested will be 0, 1, 3, 9, 27, 54 and 108 µg/L. Selection of exposure concentrations was based on previous information provided by

the USFWS (2008) and ENTRIX (preliminary data from 2008 studies), and concentrations cover the range of NOAECs, LOAECs, and LC50s established during these studies. The general activity schedule and summaries of the test acceptability requirements and test conditions for conducting the studies are provided in Tables B1-6 through B1-8, respectively. Studies will be conducted at the field laboratory at upstream field site (city water intake of the City of Trail, B.C., Canada).

B1.5 Sample Types

Two types of samples will be taken during the studies described herein:

1. Samples for biological analysis: Sturgeon will be preserved at intervals/life-stages specified above to ensure later analysis of:
    a. Gross morphological alterations (fixation in 10 percent buffered formalin)
    b. Histological analysis (fixation in 10 percent buffered formalin)
    c. Potential biochemical/molecular assessments (preservation in liquid nitrogen)
2. Samples for exposure assessment: Three different matrices will be sampled for assessment of exposure:
    a. Water: Surface water samples collected near bottom at the test system water intake at each study site, and water samples from the influx and efflux of each test system will be collected and preserved for COI, cation, anion, and DOC and TOC analysis.
    b. Tissue: Subsamples of sturgeon will be collected and preserved at four time points (Day 0, 10, 30, and at the end of study) during the course of the experiments for potential later analysis for COIs.

B1.6 Study Contingencies

The study design is intended to meet the DQOs and satisfy the data needs of the RI/FS and ecological risk assessments specific to white sturgeon. It is possible that complications may arise due to unexpectedly great mortalities in the controls (>50 percent during transition to feeding). The following is a description of possible contingencies or alternative approaches to be followed in the event control mortalities are unacceptably high. Use of contingency plans will be coordinated with EPA prior to implementation.

Contingency #1—Maintain backup batch of fish at hatchery

Arrangements with the Kootenay Trout Hatchery will be made to retain a contingent of fish from the same fertilization event at the hatchery (~3,000 fry) as a backup if mortality rates in the controls are too great to be able to reinitiate studies at a later life stage.

Contingency #2—Obtain fertilized eggs/fry from possible second spawning event

In past years, there often has been more than one spawning event at Waneta, and in such occasions ripe females and males were collected and transported to the Kootenay Hatchery at different time points for stripping and fertilization of eggs. In case there would be a second later spawning event at the hatchery in 2009, a potential second batch of eggs could be obtained for reinitiation of the experiments.

B2 SAMPLING METHODS REQUIREMENTS

B2.1 Sample Processing and Laboratory Analyses

For water quality and COI analyses current EPA analytical methods for analysis of total and dissolved metals and metalloids, conventional parameters, and nutrients and major ions will be used, in addition to *Standard Methods for the Examination of Water and Wastewater* (SM) (APHA 1998), as indicated in Table B2-1. All sample processing procedures will be entered into the appropriate forms, and dated and initialed by the person that took the sample.

Water Quality Analysis—Water quality will be either measured directly on site (temperature, pH, dissolved oxygen, conductivity, hardness, alkalinity) or appropriately stored and/or preserved for later analysis in the laboratory immediately after the sample is taken (Table B2-1). All procedures including transport of samples from the field site to the laboratory for analysis will be recorded in the COC forms that will accompany the samples at all times (UCR-SOP#3). All procedures will be in accordance with EPA methods or standard methods on a performance basis.

Samples for COI Analysis—Water samples for metal analysis will be taken and preserved as specified in Table B2-2. All procedures including transport of samples from the field site to the laboratory for analysis will be recorded on the COC forms that will accompany the samples at all times. All methods for sample preparation and fixation are in accordance with EPA methods on a performance basis.

Biological Samples—All samples will be measured and weighed immediately after sampling, and data as well as any abnormalities will be recorded on the appropriate

forms. Then samples will be transferred into appropriately labeled vials containing formalin. After 24 to 48 hours, depending on size of the individual, the sample will be transferred into ethanol, and thusly stored until further analysis. All sturgeon that die prematurely prior to termination of the experiments will be subjected to the same procedures as described in this paragraph.

In situ studies—Sturgeon egg and larvae sub-samples will be taken at six time points throughout the entire exposure duration (Figure B1-2). A subset of eggs or sturgeon at Day 0, 10, 30 and at the end or the exposure period will be frozen in liquid nitrogen for later analysis of COI concentrations in tissues.

96-Hour LC50 studies—Sturgeon will be observed after 6, 12, 24, 48, 72, and 96 hours.

The planned sampling design and rationale for selection of endpoints are detailed in the study protocols. Any modifications to the work tasks described therein will be presented as an addendum or update to these protocols.

B2.2 Sampling Documentation

Study team members will maintain bound logbooks to provide a daily record of significant events, observations, and measurements during sampling and routine experimental maintenance procedures. Each data book will have a unique identifier and each page and carbon copy will include this data book identifier. All information pertinent to sampling will be recorded in the logbooks. Each day's logbook entries will be signed and dated and will include:

- Name and title of author, date and time of entry (only on-site studies) and experimental conditions during the activity (e.g., water quality parameters, health status of test organisms)
- Activities performed (e.g., water renewal, feeding of larvae)
- Sampled matrix
- Sample collection method
- Number of samples taken.

When activity-specific data forms are used, they will also include:

- Project name and number
- Treatment identifier
- Initials

- Analysis and sample collection method.

The following information will be recorded either in the logbook or on the activity-specific data forms:

- Date and time of collection
- Sample identification number(s)
- Sample destination (e.g., laboratory)
- Field/lab observations
- Experimental measurements
- Experimental handling (preservation).

All original data recorded in experimental logbooks, data forms, sample labels, and COC forms must be written with waterproof, indelible ink. None of these accountable, serialized documents are to be destroyed or discarded, even if one is illegible or contains inaccuracies requiring document replacement. If an error is made on an accountable document assigned to one individual, that individual will make all corrections simply by crossing a line through the error, initialing and dating the correction, and entering the correct information. The erroneous information will not be obliterated. The person who made the entry will correct any subsequent error discovered on an accountable document. All personnel will be trained in the proper use of notebooks during training for work.

During the course of this study, U of S or ENTRIX will undertake to enter data into a personal digital assistant (PDA) system to aid in automation of data collation and upload. Procedures and systems for the entry, verification, backup, and compilation are currently under way. Until these systems and procedures are in place and U of S can verify the integrity and security of such data, the hard copy paper records discussed above will be kept. All PDA-entered data records will contain the same information as the paper records. When PDA data entry is implemented it will be the responsibility of the laboratory project manager to ensure that suitable electronic and/or paper copies of all PDA data are prepared and transferred to the security of the project archive.

B2.3 Sample Identification

The analysis and sample identity information are recorded in bound logbooks or recorded on data sheets while in the custody of the sampling team.

A sample label will be completed and attached to each sample container for every individual or composite sample collected. Labels consist of a waterproof material backed with a water-resistant adhesive. Labels are to be filled out using waterproof ink, and are to contain at least the following information:

- Sampling date and time
- Sample identification number
- Investigation location
- Sampler's initials
- Sample matrix or matrix identifier.

Each sample to be analyzed for residues (COIs) will be assigned a unique number consisting of an alphanumeric code that identifies the treatment group and sample type. These numbers will be tracked electronically, from collection through laboratory analysis and into the final reports.

The sample number will be cross-referenced with the site name and sample location on the COC. Additional sample volume will be collected for samples identified for laboratory QC purposes (i.e., MS, MSD, DUP) and identified as "For Lab QC Use." Information to be included on COCs is specified in SOP-UCR#1 (Appendix C), titled "Sample Management: Receiving, Preservation, Storage, Documentation, Decontamination, and Disposal."

B2.3.1 Tissue Sample Handling Procedures

Appropriate sample containers will be sealed and labeled. In cases where tissue samples will be preserved for later biochemical or chemical analysis, samples will be placed on wet or blue ice in an insulated container for not longer than 30 minutes, and then stored appropriately (e.g., liquid nitrogen). Appropriate COC documentation will accompany the samples as required by the QAPP. Specific sample volumes, sample containers, preservatives, and replication of samples are detailed in the following sections. Any sampling equipment that will be reused will be decontaminated by rinsing with deionized water followed by dilute acid (e.g., nitric acid) between sampling.

B2.3.2 Decontamination Procedures and Materials

All equipment used during investigation activities that could come into contact with chemically affected materials will be thoroughly cleaned, before and after each use, by washing with Liquinox® (a laboratory-grade detergent) and rinsing with deionized

water followed by dilute acid. Decontamination procedures may be modified and/or revised based upon the data obtained or the equipment used.

Decontamination waste is expected to consist of dilute acid. Decontamination solutions will first be discharged to drums in a designated staging area and then later transferred to laboratory facilities for proper disposal and management.

B2.4 Sampling/Measurement Failure Response

If quality control surveillance and/or experimental audits result in detection of unacceptable conditions, procedures, or data, the project manager, in conjunction with the quality assurance manager, will be responsible for developing and directing implementation of corrective actions. Corrective actions will include one or more of the following:

- Identifying the root cause of the problem and implementing systems to prevent future occurrences
- Identifying the source of the violation
- Evaluating and amending sampling and/or analytical procedures
- Accepting data and flagging the data to indicate the level of uncertainty associated with failure to meet the specified quality control performance criteria.

Any finding requiring corrective action must be documented by the project manager. The project quality assurance manager will check to ensure that corrective actions have been implemented and that the problem has been resolved. Problems will be addressed and the corrective action noted in the appropriate notebook.

If an error is made on an accountable document assigned to one individual, that individual will make all corrections simply by crossing a line through the error, initialing and dating the correction, and entering the correct information. The erroneous information will not be obliterated. The person who made the entry will correct any subsequent error discovered on an accountable document.

B2.5 Sample Preservation and Holding Time Requirements

The sample containers, preservative requirements, and maximum holding times for analytical methods used in this project are provided (Table B2-2). All containers for samples submitted for chemical analyses will have screw-type lids to ensure adequate

sealing. Commercially available, precleaned bottles will be used for chemistry samples, and the laboratory will maintain a record of certification from the suppliers.

B3 SAMPLE HANDLING AND CHAIN OF CUSTODY REQUIREMENTS

Proper sample handling, shipment, and maintenance of COC are key components of building the documentation and support for data that can be used to make project decisions. It is essential that all sample handling and sample COC requirements be performed in a complete, accurate, and consistent manner. Sample handling and custody requirements must be followed for all samples collected as part of this project.

B3.1 Sample Custody

Sample custody and documentation procedures described herein must be followed throughout all sample collection activities. Components of sample custody procedures include the use of experimental logbooks, sample labels, custody seals, and COC forms. The COC form must accompany the samples during shipment from the experimental sites to the laboratory.

A sample is under custody under the following conditions:

- It is in one's actual possession
- It is in one's view, after being in his or her physical possession
- It was in one's physical possession and that person then locked it up to prevent tampering
- It is in a designated and identified secure area.

The following procedures must be used to document, establish, and maintain custody of samples:

- A sample label will be completed and attached to each sample container for every sample collected. Labels consist of a waterproof material backed with a water-resistant adhesive. Labels are to be filled out using waterproof ink, making sure that the labels are legible and affixed firmly on the sample container. Sample labels are to contain at least the following information: sampling date and time; sample identification number; treatment group identifier; and sampler's initials.
- All sample-related information must be recorded in the project logbook or on activity-specific data forms.

- The sampler must retain custody of samples until they are transferred or properly dispatched.

To simplify the COC record and minimize potential problems, as few people as possible should handle the samples or other physical evidence. For this reason, one individual from the experiment crew should be designated as the responsible individual for all sample transfer activities. This investigator will be responsible for the care and custody of the samples until they are properly transferred to another person or facility.

A COC record will accompany all samples. This record documents the transfer of custody of samples from the investigator to another person, to the laboratory, or other organizational entities, as a signature for relinquishment and receipt of the samples must accompany each change of possession. The COC record will be prepared for groups of samples collected at a given location on a given day.

The COC form makes provision for documenting sample integrity and the identity of any persons involved in sample transfer. Information entered on the COC will consist of the following:

- Project name and number
- Logbook number
- COC serial number
- Treatment group
- Sample numbers
- Sampler/recorder's signature
- Date and time of collection of each sample
- Sample type
- Analyses requested
- Inclusive dates of possession
- Name of person receiving the sample
- Date of receipt of sample
- Name, address, and telephone number of laboratory
- Name, address, and telephone number of person to whom laboratory report will be sent

- Method of delivery and courier.

Completed COC forms will be inserted into a Ziploc® bag, sealed, and taped to the inside cover of the shipping container used for sample transport from the experimental site to the laboratory when a courier or shipping company is used. The shipping company will not sign for custody of the samples.

When samples are relinquished to a courier for transport, the tracking number from the shipping bill or receipt will be recorded on the COC form or in the site logbook.

The recipient for the samples must be notified of the date of shipment and anticipated time of arrival. The shipping bill number must also be provided to the recipient to enable tracking of samples. It must be clearly established prior to shipment who will be responsible for ensuring that timely sample delivery occurs and who will track the samples in case of shipping delays. The recipient of the samples must inform the sender when the samples are delivered. Custody seals must be affixed on shipping containers when samples are shipped to the laboratory to prevent sample tampering during transportation. In cases of delivery delay or packing damage all details of damage and sample condition must be recorded and if necessary photographed for documentation.

B3.1.1 Laboratory Sample Handling and Custody

The project liaison or study team leader will notify the laboratory project manager of upcoming sampling activities and the subsequent transfer of samples to the laboratory. This notification will include information concerning the number and type of samples to be shipped, analyses requested, and the expected date of arrival. The laboratory project manager will notify appropriate laboratory personnel about the expected shipment including the sample custodian.

Upon arrival at the laboratory, the samples will be received and logged in by a trained sample custodian in accordance with the laboratory's sample handling program. A description of the laboratory's general program is provided in SOP-UCR#2 & 3 and is summarized below.

Upon sample receipt, the sample custodian is responsible for performing the following activities during sample receipt where appropriate:

- Examining the shipping containers to verify custody seals, if used, are intact
- Examining all sample containers for damage
- Taking digital photographs of any custody seals used, before opening, and of any damage to the shipping container or individual sample containers

- Comparing samples received against those listed on the COC
- Verifying sample holding times have not been exceeded
- Determining sample temperature (from the temperature blank vial) and documenting variations from the acceptable range on the COC
- Verifying that all samples listed on the COC are present or accounted for
- Immediately signing and dating COC after shipment is accepted
- Noting any sample receipt problems on the COC, initiating a condition-upon-receipt (CUR) report, and notifying the laboratory project manager
- Attaching laboratory sample container labels with laboratory identification number and test
- Placing the samples in proper laboratory storage.

The laboratory project manager is responsible for contacting the project liaison as soon as possible if any problems are identified during sample receipt. All identified sample receiving problems will be resolved before sample preparation and analysis.

Following sample receipt, the sample custodian is responsible for logging the samples in the laboratory sample log-in book, and/or the Laboratory Information Management System with the following information:

- Laboratory project number
- Sample numbers
- Type of samples
- Required tests
- Date collected
- Date received.

The sample custodian is also responsible for notifying the laboratory project manager and appropriate group/team leader(s) of sample arrival and placing completed COCs, waybills, and any additional documentation in the project file.

Samples will be stored appropriately within the laboratory to maintain any prescribed temperature, to protect against contamination, and to maintain the security of the samples.

If any samples are transferred to a different laboratory, the transfer will be done under COC procedures, and ENTRIX will maintain the appropriate documentation to preserve the traceability of the samples through final analysis and disposal.

B3.2 Sample Packing and Shipping

Samples will be delivered to the designated laboratories by experimental personnel, laboratory courier, or by commercial shipping services (such as UPS or Federal Express). The method of sample shipment will be noted on the COC. During the experimental effort, the experiment team leader or a designee will inform the laboratory daily of planned shipments. Hard plastic ice chests or coolers with similar durability will be used for shipping samples. The coolers must be able to withstand a 4-ft drop onto solid concrete in the position most likely to cause damage. The samples will be packed to prevent the least amount of damage if such a fall were to occur.

After packing is complete, the cooler will be taped shut with custody seals affixed across the top and bottom joints. Each container will be clearly marked with a sticker containing the originator's address.

The following procedures must be used when transferring samples for shipment.

- A COC form must accompany samples. When transferring possession of samples, the individuals relinquishing and receiving must sign, date, and note the time on the record. This record documents transfer of custody of samples from the sampler to another person or to the laboratory. Overnight shipping companies will not be required to sign the COC. A copy of the receipt of shipment will accompany the COC.
- Samples must be properly packaged for shipment and dispatched to the appropriate laboratory for analysis with a separate signed COC form enclosed in each sample box or cooler. The COC should reflect *only* the contents of the cooler in which it is enclosed.
- A COC form identifying the contents must accompany all packages. The original record must accompany the shipment, and the field team leader must retain a copy.

B4 ANALYTICAL METHODS REQUIREMENTS

This subsection presents the analytical methods requirements for analyses that may be performed during the study including preparation/extraction procedures where appropriate and method performance requirements.

The Environmental Toxicology Laboratory at the U of S will conduct laboratory analyses of biological materials. Analysis of samples for COIs will be conducted by Columbia Analytical Services. The laboratory's quality assurance protocols will be available in the project files and will contain summary information from the analytical methods including the following:

- Sample containers, preservatives, and holding times
- Calibration requirements including frequency and acceptance criteria
- Laboratory quality control samples including frequency, acceptance criteria, and corrective action
- Limits of detection.

More detailed information on the laboratory's analytical methods is presented in laboratory-specific SOPs and the 2008/2009 surface water study QAPP.

B4.1 Analytical Methods

B4.1.1 Biological Assessments

Hatchability—Hatchability will be assessed 72 hours post fertilization. Sub-samples of 100 eggs per replicate treatment will be evaluated for completed neurolation, which has been a reliable indicator for successful hatching in white sturgeon eggs (Kootenay Trout Hatchery, pers. comm.). Based on replicate measurements of the percentage of completely neurolated eggs, the overall hatchability for each treatment group will be assessed. Results will be presented as percent hatchable eggs.

Mortality—Mortality data will be reported as the percentage of dead embryos or fry/juveniles compared to original seeding density adjusted for thinning. Mortality will be assessed as follows: 1) Embryo mortality: Unfertilized eggs and/or dead embryos are discerned from live ones by change in coloration and opacity. At later stages, lack of heartbeat and movement observable through the chorion are indicators of mortality. 2) Fry/juvenile mortality: Dead fish will be discerned from live ones by immobility (e.g., in response to gentle prodding) and absence of respiratory movement in older individuals.

Growth—Growth will be measured throughout the duration of the experiment by measuring the fish to the nearest 0.5 mm and the nearest 0.01 g. Individuals will be blotted dry prior to determining weight. The determination will be done on fish removed for thinning purposes at the interval shown in Figure B1-2. Growth will be reported as weight or length gain over time.

Other observations—Throughout the experiment, fish will be monitored for alterations in behavior (e.g., lethargy or hyperactivity). Furthermore, animals will be inspected for gross morphological alterations (e.g., fin aberrations, skeletal deformities) at the time of sampling. All individuals removed from the experiments will be fixed in formalin for possible later histological analysis.

B4.1.2 Chemical Analysis

Near-bottom water samples will be analyzed for dissolved and total metals and metalloids, conventional parameters, nutrients, and major ions. Consistent with the DQOs identified in Section A7 of the 2008/2009 surface water study QAPP, the analytical concentration goals are less than conservative benchmarks and literature-derived values for aquatic and terrestrial ecological receptors and human health. To determine the reporting limit goals, available guidelines and historical reporting limits were compiled and compared to the expected reporting limit. For aquatic ecological receptors, reporting limit goals were developed using the EPA National Aquatic Life Chronic Criteria (USEPA 2006b), Colville Confederated Tribes Aquatic Life Chronic Criteria (40 CFR 131.35), the Ecology Aquatic Life Chronic Criteria (WAC 173-201A), and the Spokane Tribe of Indians Aquatic Life Chronic Criteria (Spokane Tribe of Indians 2003). Reporting limits from Paulson et al. (2006) were also tabulated because they include metals not routinely analyzed and for which screening ecological values are lacking.

The screening values and required method reporting limits (MRLs) for samples collected during the 2009 sturgeon ELS studies are provided in Appendix D. The goal is for MRLs from the analytical laboratories to be equal to or below one-fifth of the lowest screening value for each analyte. MRLs are generally equivalent to the concentration of the lowest calibration standard (i.e., the practical quantification limit) and represent the low end of the calibration range. Analytes that are detected at concentrations below the reporting limit but above the detection limit will be reported, but will be qualified as estimated (i.e., a "J" qualifier will be applied to the result by the laboratory).

Laboratory methods for sample preparation and analysis are summarized in Table B2-1, and are described in the following sections. Sample containers, preservation, and holding times are provided in Table B2-2.

Total and Dissolved Metals

Standard metals and metalloids (EPA target analyte list metals), molybdenum, and uranium will be analyzed in all samples collected at all of the study sites.

Three methods will be used to analyze samples for total and dissolved metals and metalloids (see Table B2-1). Digestion with nitric and hydrochloric acids will be used to prepare samples for analysis of metals other than mercury. Analysis for these metals and metalloids will be completed by inductively coupled plasma/mass spectrometry (ICP/MS) and inductively coupled plasma/atomic emission spectrometry (ICP/AES), according to EPA Methods 6020 and 6010B, respectively.

Mercury samples will be oxidized with the addition of bromine chloride and analyzed by stannous chloride reduction, followed by gold amalgamation, thermal desorption, and atomic fluorescence spectroscopy according to EPA Method 1631.

Conventional Parameters

Conventional parameters that will be analyzed in the near-bottom water samples will include alkalinity as $CaCO_3$, DOC, hardness as $CaCO_3$, total dissolved solids (TDS), total suspended solids (TSS), TOC, pH, and silica as dissolved SiO_2. *Standard Methods for the Analysis of Water and Wastewater* (SM) (APHA 1998) will be used, as shown in Table B2-1.

Alkalinity and hardness as $CaCO_3$ will be determined titrimetrically according to SM 2320B and 2340C, respectively. TDS and TSS will be determined gravimetrically according to SM 2540.

TOC and DOC will be analyzed by SM 5310C; organic carbon in near-bottom water samples will be oxidized and the evolved carbon dioxide will be analyzed using an infrared detector.

Nutrients

Nutrients to be analyzed in near-bottom water samples include ammonia as nitrogen, nitrate, nitrite, and total phosphorus. EPA and SM methods will be used as shown in Table B2-1.

Nitrate and nitrite as nitrogen will be determined by ion chromatography according to EPA Method 300.0.

Ammonia as nitrogen will be determined colorimetrically according to SM 4500-NH3 G. Total phosphorus will be determined colorimetrically according to EPA Method 365.3.

Major Ions

Major ions to be analyzed in near-bottom water samples include calcium, magnesium, potassium, sodium, chloride, fluoride, and sulfate. EPA and SM methods will be used as shown in Table B2-1.

Chloride, fluoride, and sulfate will be determined by ion chromatography according to EPA Method 300.0.

Samples being analyzed for calcium, magnesium, potassium, and sodium will be digested with nitric and hydrochloric acids and analyzed using ICP/AES, according to EPA Method 6010B.

On Site Measurements

In addition to sample collection for chemical analysis at the testing laboratory, a number of general water quality parameters (i.e., water temperature, pH, dissolved oxygen, conductivity, ammonia, nitrate, nitrite, hardness) will be routinely measured *in situ* in all recirculating systems.

B4.2 Laboratory Corrective Action

Laboratories have formal corrective action systems in place to ensure that prompt action is taken when an unplanned deviation from a procedure or plan occurs and that whenever possible, corrective actions include measures to prevent the reoccurrence of deviations. Specific corrective actions will be taken and documented when a quality control sample does not meet acceptance criteria. Following is a description of how information from the laboratory's corrective action system is communicated to the project team.

Corrective action procedures include prompt notification of the project contact (quality assurance manager) for any significant problems or discrepancies. The laboratory project manager is responsible for reporting any significant problems or discrepancies that occur as analyses are conducted to the project liaison or other identified project contact. The laboratory project manager is also responsible for ensuring that corrective action is taken where appropriate to prevent the reoccurrence of similar problems or discrepancies. In addition, each analytical data report will include a case narrative that discusses any problems or discrepancies, and sufficient calibration and quality control information to verify that the method was in control at the time that the samples were analyzed. The case narrative will also include a discussion of any corrective action taken by the laboratory to prevent the reoccurrence of similar problems or discrepancies.

B5 EXPERIMENTAL QUALITY CONTROL REQUIREMENTS

This section presents the experimental and analytical quality control checks that will be performed during investigations including a discussion of quality control samples with frequency and acceptance criteria and corrective action procedures.

B5.1 Quality Control Samples

Quality control samples will be prepared in the field and at the laboratories to monitor the bias and precision of the sample collection and analysis procedures. The type and frequency of quality control samples to be collected during investigations are summarized in Table B5-1 and are described below:

B5.1.1 Equipment Rinsate Blank Samples

Equipment rinsate blanks (ERBs) are samples of weak acid (e.g., 1 percent nitric acid) passed through and over the surface of decontaminated sampling equipment. The rinsate is collected in sample bottles, preserved, and handled in the same manner as the samples. ERBs are used to monitor effectiveness of the decontamination process. The planned frequency for ERBs is one per week per equipment type. If more than one type of equipment is used to collect samples for a particular matrix, then an ERB is collected and submitted for each representative group of equipment. Typically, ERBs are analyzed for the same analytes as the corresponding samples collected that day.

B5.1.2 Blanks

Blanks are unopened sample containers which are transported to and returned from the experimental location. Typically, at least one blank per lot number of collected samples will be analyzed.

B5.1.3 Duplicate (Blind) Samples

"Blind" duplicate samples are collected to monitor the precision of the sampling process. The use of replicates to assess precision is discussed in Section B5.2.1. Appropriate experimental duplicates will be collected and submitted to the laboratories for analysis.

B5.2 Method Performance Objectives

Method performance requirements for analytical laboratory methods to be performed for the study are expressed in terms of precision, accuracy, representativeness, comparability, completeness, and sensitivity (PARCCS). Summarized below are brief definitions for each PARCCS parameter, with calculation equations as appropriate.

B5.2.1 Precision

Precision is an estimate of the variability between individual measurements of the same physical or chemical property, under prescribed similar conditions.

Experimental Precision

Experimental precision is usually assessed through the collection and measurement of duplicate samples from each treatment. The duplicate sample is submitted "blind" to the laboratory, and sample results are compared to check for the overall variability introduced by sampling and analytical procedures. The experimental duplicate approach is generally not applicable to systems where the experimental unit is the single organism because each individual represents a sampling replicate. Similarly, when a single test solution sample is collected and divided into additional blind samples, these replicate samples represent analytical replicates.

Analytical Precision

Precision in the laboratory is assessed through the calculation of the relative percent difference (RPD) for two replicate samples. The precision of the analysis can be inferred through the use of one of the following: 1) standard reference materials (SRMs) and duplicate SRM (SRMD) samples; 2) matrix spike and matrix spike duplicate (MS/MSD) samples which are project samples spiked with known analyte concentrations; or 3) duplicate analyses of unspiked project samples. The laboratory analyzes one or more of the aforementioned types of duplicate samples at a rate of one per batch of 20 or fewer investigative samples per matrix.

The MS/MSD samples provide information about the effect of the sample matrix on extraction and measurement methodology. An MS/MSD pair will be analyzed at a rate of one per 20 per analytical batch or fewer investigative samples per matrix.

Calculating the RPD for each pair of duplicate analyses (e.g., MS/MSD, LCS spike duplicates, unspiked duplicate samples) and the RPD for experimental duplicate sets, using the following formula will assess the precision of laboratory analyses:

$$RPD = \frac{S - D}{(S + D)/2} x100 \qquad \text{(Eq. B5-1)}$$

Where:

RPD = Relative percent difference
S = First sample value (original or MS value or larger of the duplicate)

D = Second sample value (duplicate or MSD value or smaller of the duplicate)

B5.2.2 Accuracy

Accuracy is the degree of agreement between a measurement or observation and an accepted value.

Experimental Accuracy

Experimental accuracy is assessed through the collection and analysis of appropriate experimental blanks, and achieved through adherence to all sample handling, preservation, and holding time requirements. Experimental blank samples are analyzed to check for procedural contamination that may cause sample contamination. Equipment rinse blanks are used to assess the adequacy of decontamination of sampling equipment between collections of individual samples. Accuracy of instruments will be assessed by using weekly instrument calibration and calibration checks. Experimental blank and equipment rinsate blank analysis frequencies are given in Table B5-1.

Analytical Accuracy

Laboratory accuracy is assessed by the analysis of method blanks and matrix spikes, LCS, and/or SRM or certified reference materials. The results are expressed as percent recovery. Method blank samples are generated within the laboratory and used to assess contamination resulting from laboratory procedures. Surrogate compounds are used in analyses for inorganic contaminants and are specified in the analytical methods described in the 2008/2009 surface water study QAPP.

Method blanks, matrix spike, LCS, and/or SRM samples will be analyzed at a rate of one per analytical batch of 20 or fewer investigative samples/matrix.

The percent recovery (percent R) of spike samples will be calculated using the formula:

$$R = \frac{A - B}{C} x100 \qquad \text{(Eq. B5-2)}$$

Where:

R = Recovery (percent)

A = The analyte concentration determined experimentally from the spiked sample, units

B = The background level determined by a separate analysis of the un-spiked sample, units

C = The amount of the spike added, units.

B5.2.3 Representativeness

Representativeness is a qualitative measure of the degree to which sample data accurately and precisely represent a characteristic experimental condition. Representativeness is a subjective parameter and is used to evaluate the efficacy of the study plan design. Representativeness is demonstrated by providing full descriptions of the sampling design and its rationale in the project planning documents.

There cannot be a target numerical goal for a qualitative parameter such as representativeness or comparability. Therefore, this criterion is completed and evaluated subjectively rather than quantitatively. The measure for representativeness is answered during the preparation of the sampling and analysis approach and rationale, and then reassessed during the data usability process. For example, an integral part of developing the sampling and analysis approach and rationale is to answer the question "How many samples are needed to fully evaluate x?" Then, during the data usability process, the question "Were enough data collected to answer the original question?" must be answered. Thus, it is not possible to construct a table with numerical goals that can be used to evaluate these subjective measures. The criteria to make these decisions can be based on power analysis conducted after initial information has been collected or during data interpretation to determine if additional samples are necessary to fully describe the nature and extent.

B5.2.4 Comparability

Comparability expresses the confidence with which one data set can be compared with another data set obtained during parallel or previous investigations. Comparability can be related to precision and accuracy, since these parameters are measures of data reliability.

Results are generally considered comparable if the same procedures for collecting and analyzing the samples are employed, if the samples comply with the same QA/QC procedures, and if the units of measurements are the same.

The study protocols for the determination of biological effects for this study were designed such that the data obtained during these studies are comparable with data collected during previous studies as outlined in Section A5 where applicable. Furthermore, comparability will be assessed by the parallel assessment of four true replicates for each treatment in the experiments.

The quality objectives for data from the exposure experiments and analytical tasks within this study is to achieve a level of comparability that allows for the comparison of

data collected within and among all experiments. To accomplish this goal, all data generated during the tasks included in this investigation will be subject to strict QA/QC procedures as specified in this QAPP. Furthermore, comparability will be assessed by including a separate control using laboratory water in the field. Key water quality parameters known to influence availability and toxicity of metals and/or that are of importance for larval development and growth will be adjusted in the acute toxicity studies for comparability reasons. These parameters include temperature, pH, hardness, and dissolved oxygen.

B5.2.5 Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was planned to be obtained under normal conditions. Data completeness will be calculated by using Equation B5-3.

$$\%\,\text{Completeness} = \frac{\text{Valid Data Obtained}}{\text{Total Data Planned}} \text{x} 100 \qquad \text{(Eq. B5-3)}$$

Experience on similar projects has shown a reasonable goal considering combined historical field and laboratory performance is 90 percent completeness. All valid data will be used. During the data validation process, an assessment will be made of whether the valid data are sufficient to meet project objectives. If sufficient valid data are not obtained, the project manager will initiate corrective action. Where invalid data are generated, all documentation and the reasons for the invalidation of the data will be provided.

B5.2.6 Sensitivity

Sensitivity is the measure of the concentration at which an analytical method can positively identify and report analytical results. The sensitivity of a given method is commonly referred to as the detection limit. Although there is no single definition of this term, the following terms and definitions of detection limits will be used for this project:

Instrument detection limit: Defined as the minimum mass of analyte that can be measured above instrument background noise under ideal conditions.

Analytical detection limit: Method detection limits (MDLs) are statistically derived and reflect the concentration at which an analyte can be detected in a clean matrix with 99 percent confidence that a false positive result has not been reported. The laboratory conducting the analysis will determine a method detection limit for each analyte, as required by USEPA (2004). The analytical laboratory will have established MRLs at

levels above the MDLs for the task analytes. These values are based on the laboratory's experience analyzing environmental samples, reflect the typical sensitivity obtained by the analytical system, and represent the level of analyte above which concentrations are accurately quantified. Analyte concentrations for this study will be reported to the MDL. Analytes detected at concentrations between the MRL and the MDL will be reported with a "J" qualifier to indicate that the value is an estimate (i.e., the analyte concentration is below the calibration range). Nondetected values will be reported at the MRL and will be adjusted by the laboratory as necessary to reflect sample dilution or matrix interference.

B6 EQUIPMENT INSPECTION, AND MAINTENANCE REQUIREMENTS

Maintenance and inspection of both experimental and analytical equipment are described in the following sections.

B6.1 Experimental Instruments/Equipment

Preventive maintenance of instrumentation and equipment will be performed according to manufacturer's instructions. The onsite staff is responsible for ensuring that all instrumentation is operating properly prior to use. If problems are encountered, they will be documented in a bound notebook. The faulty instrumentation/equipment will be scheduled for repair and sequestered and tagged until repaired and qualified for reuse.

B6.2 Analytical Instrument/Equipment

Analytical instrument/equipment testing, inspection, and maintenance will be conducted in accordance with the procedures specified in the manufacturers' directions. The quality assurance manual discusses the schedule, procedures, criteria, and documentation in place at the laboratory to prevent instrument and equipment failure and to minimize downtime. For each instrument or piece of equipment, the laboratory maintains the following:

- Instrument/equipment inventory list
- Instrument/equipment major spare parts list or inventory
- External vendor service agreements (if applicable)
- Instrument-specific preventive maintenance logbook or file.

The laboratory documents all preventive maintenance and repair for each instrument or piece of equipment in dedicated logbooks or files.

B7 INSTRUMENT CALIBRATION AND FREQUENCY

Calibration and frequency of calibration of both experimental and analytical equipment are described in the following sections.

B7.1 Experimental Instruments

The experimental equipment that will need calibration is listed below:

- Water quality meter
- Balance
- Pumps.

Proper maintenance, calibration, and operation of each instrument will be the responsibility of experiment personnel assigned to a particular activity. All instruments and equipment used during the investigations will be maintained, calibrated, and operated according to the manufacturer's guidelines and recommendations. If an individual suspects an equipment malfunction, the device must be removed from service and tagged so that it is not inadvertently used, and the appropriate personnel notified so that a recalibration can be performed or a substitute piece of equipment can be obtained. An extra or backup meter will be taken into the field to replace the inoperable unit.

Results of measurements performed using equipment that has failed recalibration will be evaluated. If the measurement results are adversely affected, the results of the evaluation will be documented, the data qualified appropriately, and the data users notified.

B7.2 Analytical Equipment and Instrumentation

All laboratory equipment and instruments used for quantitative measurements are calibrated in accordance with the laboratory's formal calibration program as described in the quality assurance manual. A summary of the laboratory instrument/equipment calibration program is presented in that manual. Detailed calibration procedures specific to each analysis are included in method-specific SOPs, which can be obtained from the laboratory.

Whenever possible, the laboratory uses recognized procedures for calibration such as those published by EPA or ASTM. If established procedures are not available, the laboratory develops a calibration procedure based on the type of equipment, stability, characteristics of the equipment, required accuracy, and the effect of operation error on

the quantities measured. Equipment requiring only periodic calibration such as pumps, balances, thermometers, and micro-pipetters are listed along with their respective calibration requirements in the quality assurance manual. Whenever possible, physical reference standards associated with periodic calibrations such as weights or certified thermometers with known relationships to nationally recognized standards are used. Where national reference standards are not available, the basis for the reference standard is documented.

Other instruments that require initial and/or continuing calibration as a part of instrument usage are listed along with their respective calibration requirements in the quality assurance manual. Initial calibrations are verified and documented for each constituent by analysis of laboratory-prepared certified independent standard solutions.

All calibration standards will be obtained from either the EPA repository or a commercial vendor, and the laboratories will maintain traceability back to the National Institute of Standards and Technology. Stock standards will be used to make intermediate standards and calibration standards. Special attention will be given to expiration dating, proper labeling, proper refrigeration, and prevention of contamination. Documentation relating to the receipt, mixing, and use of standards will be recorded in a laboratory logbook. All calibration and spiking standards will be checked against standards from another source, as specified in the methods and the laboratory quality assurance manual.

B8 ACCEPTANCE REQUIREMENTS FOR SUPPLIES AND CONSUMABLES

Supplies and consumables that may be used during the investigations include sample bottles, petri dishes for filter storage, hoses, filters, nitric acid, formalin, ethanol, materials for decontamination activities, potable water, deionized water, and ASTM Type II water, MS 222, *Artemia salina* eggs marine salts, bloodworms, and water quality test kits. Project team members obtaining supplies and consumables are responsible for assuring that the materials obtained meet the required specifications, are intact and in good condition, are available in adequate supply, and are stored appropriately until use. Project team members will direct any questions or identification of any problems regarding supplies and consumables to the experiment team leader for resolution.

B9 NON-DIRECT MEASUREMENTS

Existing data from previous studies will be used for this study (see Section A5). As discussed in the RI/FS work plan, historical data will be reviewed for quality assurance and acceptability prior to use in the RI/FS.

B10 DATA MANAGEMENT

Data management procedures will be established and applied during the investigations to record, document, track, and compile investigative data into an overall project database. Data generated during the investigations, as well as historical data, will be used to form the basis for conclusions and recommendations. Efficient utilization and comprehensive consideration of available data requires that the data be properly organized for review. Organization of the data shall be planned prior to actual collection to assure the generation of identifiable and usable data. This section contains procedures necessary to assure the collection of sufficient data for accurate validation of raw data and transfer of validated data to the project data management system. This section also describes the operating practices to be followed by personnel during the collection and reporting of data.

B10.1 Purpose and Background

Data collected during the investigations will include analytical chemistry data from water samples, and data on hatchability, survival, growth and development of ELS of white sturgeon in each treatment group. These data will be used for an analysis of the toxicity of near-bottom water in the UCR stretch of interest to these life stages, and the potential contribution of COIs to this toxicity. Data will be collected, managed, and stored in a way that models the inherent structure of the data and facilitates its usage for the RI/FS.

B10.2 Data Recording

Observations made and measurements taken during toxicity testing experiments will be recorded using appropriate hard copy (e.g., data sheets or logbooks) or electronic formats (e.g., laboratory electronic deliverables). Data recorded in hard copy will be transcribed into electronic forms and proofed before use or integration with other data.

A variety of manually entered and electronic instrument data are generated at each analytical chemistry laboratory. Data are manually entered into:

- Standard logbooks
- Storage temperature logs
- Balance calibration logs
- Instrument logs
- Sample preparation and analysis worksheets
- Maintenance logs
- Individual laboratory notebooks
- Results tables for conventional analyses (e.g., grain-size distribution, percent moisture).

All data manually entered into the laboratory information management system will be proofed at each laboratory prior to being released. All data collected from each laboratory instrument, either manually or electronically, will be reviewed and confirmed by analysts before reporting. A detailed description of procedures for laboratory data management and data review and verification is provided in the laboratory quality assurance plans.

Laboratory data will be entered directly into the project database from the electronic data deliverable (EDD). ENTRIX will perform a comparison of electronic data with the hard copy report prior to submittal to ensure that the EDD and hard copy data are identical. EDDs will be checked against the hard copy with 100 percent QA/QC for all detected analytes and other data where appropriate. The EDD should be submitted on a CD-ROM, with the disk label including the laboratory delivery group, submittal date, laboratory name, and site description. If the EDD is resubmitted, the EDD will be labeled as "Revised."

Toxicity testing and analytical chemistry data will be entered into the project database management system (DBMS) and tabulated for evaluation and presentation in the investigation report. Copies of the original data records will be attached to the report as appendices.

All data used for meeting project objectives will be stored in an electronic database. This database will facilitate the following processes:

- Tracking COC and sample identification data
- Reviewing and evaluating analytical data against project-specific QAPP criteria

- Production of data tables.

B10.3 Data Validation

Data validation is an integral part of the quality assurance program and consists of reviewing and assessing the quality of data. Data validation provides assurance that the data are of acceptable quality as reported. For validity, the characteristics of importance are precision, accuracy, representativeness, comparability, and completeness. Data usability is the determination of whether or not a data set is sufficiently complete and of sufficient quality to support a decision or action, in terms of the specific DQOs.

The data validation process includes:

- Evaluating against blank criteria
- Evaluating against accuracy criteria such as holding times, surrogates, LCS, and matrix spikes
- Evaluating against precision criteria such as MS/MSDs, and experimental and analytical duplicates
- Confirming that data qualifiers are assigned appropriately
- Uploading sample data only to the central database.

The data validation process is described more fully in Section D.

B10.4 Data Transformation

If data transformation is performed for this study, then conversion procedures will be described in detail in the associated technical report.

B10.5 Data Transmittal

Entering the data from forms into the DBMS completes the integration of data by data entry personnel. A staff scientist will review the data for completeness and accuracy by comparing the values to the original data.

Laboratory data are provided in both a hard copy and in EDD format. The electronic data are provided in a specified format that will be uploaded to intermediate files, reviewed for completeness and accuracy by the project liaison before uploading to the project DBMS.

B10.6 Data Analysis

Data analysis (e.g., computation of summary statistics, standard errors, confidence intervals) will be conducted for this project.

B10.7 Data Tracking

The project manager is ultimately responsible for all activities conducted during experimental activities, including data management. The project manager has the authority to enforce proper procedures as outlined in this plan and to implement corrective procedures to assure the accurate and timely flow and transfer of data. The project manager will review the final data reports.

Data will be generated from the observations made during the course of the experiments and during sampling and analysis activities. The generators of data will be responsible for accurate and complete documentation of data required under the task, and for ensuring that these data are presented to their supervisor in a timely manner.

The study team leader or his designees will be responsible for the day-to-day monitoring of data during the conduct of the experiments. They ensures that data are collected in the format specified in this QAPP and route data to ENTRIX to be placed in the project files at the end of the experimental activities. Original documents will be maintained in the ENTRIX central project file.

The study team leader will also be responsible for evaluating biological data. The study team leader or his designees review biological data for accuracy and completeness. The project manager will assure that representations of current experimental conditions are accurate and complete for each component of the study.

The project liaison will be responsible for the day-to-day monitoring of activities related to the generation and reporting of chemical data. The project liaison ensures that samples are analyzed according to the specified procedures; that data are validated; and that the data are properly coded, checked for accuracy, and entered into the data management system. The project liaison ensures the data are then routed to ENTRIX to be placed in the project files.

B10.8 Data Storage and Retrieval

A project file will be established for the storage of original data, historical data, written documents, and data collected or generated during the experiments. The format for the

file will follow the central filing system procedure list, which consists of the following categories:

- Correspondence
- Budgets
- Contracts
- Experimental data
- General data
- Notes/comments
- Raw data
- Figures and maps
- Permits
- Paper and electronic copies of data collected – both paper and PDA data
- Laboratory data and QA/QC documents
- Chains of custody
- Photographs
- Reports
- Schedules
- Background

All materials will be dated, carry the initials of the person responsible for the preparation of the document, and bear the project number. The file copies will include peer review sign-off on the calculation sheets and editing review sheets where applicable.

Access to the project files will be limited to those personnel assigned to this project. The project manager maintains overall responsibility for the project files and ensures that appropriate documents are filed. All documents relating to the project shall be controlled to ensure proper distribution, filing, and retrieval. The project manager will also ensure that revisions are properly recorded, distributed, and filed. ENTRIX staff maintain the project files.

ENTRIX staff will handle all documents submitted to the project file and will ensure that the documents are appropriately filed by category and placed in the correct project file.

Once filed, documents are available to ENTRIX staff and may be removed from file for use by signing out the material.

SECTION C: ASSESSMENT AND OVERSIGHT

This section presents the internal and external checks (assessments) that have been built into this project to ensure that:

- Elements of this QAPP have been correctly implemented as prescribed for all investigations conducted
- The quality of the data generated is adequate and satisfies the DQOs that have been identified in this QAPP
- Corrective actions, when needed, are implemented in a timely manner and their effectiveness is confirmed.

Assessment activities may include surveillance, inspection, peer review, management systems review, readiness review, technical systems audit, performance evaluation, and data quality assessment.

C1 ASSESSMENT ACTIVITIES

The following subsections identify the planned assessment and oversight activities to ensure the objectives identified above are attained for experimental and analytical operations. The quality assurance manager and/or the project manager may also identify additional assessment activities to be performed during the course of the project based upon findings of the planned assessment activities described below.

C1.1 Assessment of Experimental Operations

The quality assurance manager and/or other designated members of the project team will conduct internal assessments of experimental operations, where appropriate. The assessment activities will evaluate experimental operations performance issues such as:

- Are sampling and monitoring operations being conducted in accordance with the QAPP?
- Are the sample labels being filled out completely and accurately?
- Are the COC records complete and accurate?
- Are the experimental notebooks being filled out completely and accurately?
- Are the sampling and monitoring activities being conducted in accordance with SOPs?

Planned assessment activities to evaluate these and other experimental operations performance issues include surveillance (frequent review) of sample collection documentation, sample handling records (COC forms), experiment notebooks, and study measurements, and the performance of unannounced experimental operations audits.

The team member conducting the assessment activity will report the results of any assessment activities to the project manager. Assessment activity reports will include the findings and identification of any corrective actions taken or planned.

C1.2 Assessment of Analytical Operations

The project liaison will be in contact with the project manager on a weekly basis while samples collected during this investigation are being analyzed. This will allow assessment of progress in meeting DQOs and the identification of any problems requiring corrective actions early in the investigative process. The project liaison will promptly report problems identified, corrective actions taken, and recommendations as appropriate for additional corrective action to the project manager. The project manager will review the problem and provide for the swift implementation of any outstanding corrective actions. In addition, contact between the project quality assurance manager and the independent data auditor could result in the need for a laboratory audit. The project quality assurance manager will report the audit findings and any recommendations for corrective action to the project manager, the project liaison, and the laboratory. The project liaison will be responsible for working directly with the laboratory to ensure the prompt resolution of any problems identified.

C2 REPORTS TO EPA

As required by the Agreement, validated data will be provided electronically to EPA within 90 days of completion of receipt of all laboratory data packages for each survey. These data will be provided with a field sampling report containing an overview of the field event, a sampling location map, sample collection methods used, rationale for any deviations from the field sampling plan and QAPP, validated data and data validation report, and if appropriate, recommendations for changes to the sampling design for upcoming surveys.

A final data evaluation report will be prepared by ENTRIX in consultation with the Teck technical team and submitted to EPA within 150 days following submission of the third (i.e., final) field sampling report.

SECTION D: DATA VALIDATION AND USABILITY

Data generated in the field and at the laboratories will be verified and validated according to criteria and procedures described in this section. Data quality and usability will be evaluated, and a discussion will be included in the data validation report. Implementation of this section will determine whether the data conform to the specified criteria, thus satisfying the project objectives.

D1 DATA REVIEW, VERIFICATION, AND VALIDATION

Data validation is the process of reviewing data and accepting, qualifying, or rejecting data on the basis of sound criteria using established EPA guidelines. The laboratory will report laboratory data generated during the investigations in from of data packages. All of these data will be subjected to full data validation conducted by an independent data validator as discussed below in Section D1.1.

D1.1 Independent Data Validation Protocols

While the actual procedures used will be determined by the validator, the validation approach will consist of a systematic review of the analytical results, associated quality control methods and results, supporting data, and biological observations and measurements. Specific data package review procedures can be found in SOP-UCR#10, "Data Package Review," included as Appendix C in this QAPP. Best professional judgment in any area not specifically addressed by EPA guidelines will be utilized as necessary and described in the usability assessment portion of the data validation report.

Where applicable and/or appropriate, data will be validated according to applicable guidelines set forth in the following sources and guidelines to ensure compliance with the Federal Information Quality Act:

- "Data package review," SOP-UCR#10 (ETL-SOP#4039). Environmental Toxicology Laboratory, Toxicology Centre, University of Saskatchewan, Saskatoon, SK S7N 5B3.
- "Guidance for data usability in risk assessment (Part A)," EPA Publication 9285.7-09A, U.S. Environmental Protection Agency, April 1992.
- "Guidelines for ensuring and maximizing the quality, objectivity, utility, and integrity of information disseminated by the Environmental Protection Agency," EPA Publication EPA/260R-02-008, U.S. Environmental Protection Agency, October 2002.

- "Guidelines for ensuring and maximizing the quality, objectivity, utility, and integrity of information disseminated by Federal Agencies." Federal Register, 67, No. 36, pp8451-8460, February 22, 2002.
- "Guidance on environmental data verification and validation." EPA Publication QA/G-8, U.S. Environmental Protection Agency, 2002.
- "USEPA contract laboratory program national functional guidelines for inorganic data review," U.S. Environmental Protection Agency, 2004.

Data validations will include a data completeness check of each data package, a transcription check for sample results, and a thorough review of all laboratory reporting forms and the associated raw data for QA/QC issues. Specifically, this review will include:

- Review of data package completeness
- Review of the required reporting summary forms and all associated raw data to determine if the quality control requirements were met and to determine the effect of exceeded quality control requirements on the precision, accuracy, and sensitivity of the data
- Review of the overall data package to determine if contractual requirements were met
- Review of raw data and all calculations associated between one and a minimum of 10 percent of all samples to determine if the sample results and quantification limits were correctly calculated and reported
- Review of additional QA/QC parameters, such as blank contamination, to determine technical usability of the data
- Application of standard data quality qualifiers to the data.

In addition, each data validation will include a comprehensive review of the following QA/QC parameters:

- Holding times (to assess potential for degradation that will affect accuracy)
- ICP/MS instrument check (to assess accuracy and sensitivity of method)
- Initial calibration (to assess method sensitivity)
- Continuing calibration (to assess method sensitivity)
- Blanks (to assess contamination for all compounds)

- System monitoring compounds (to assess method accuracy)
- MS/MSD or laboratory fortified blanks (to assess accuracy of the methods and precision of the method relative to the specific sample matrix)
- Internal standards (to assess method accuracy and sensitivity)
- Target compound identification
- Compound reporting limit and MDL (to assess sensitivity as compared to project-specific requirements)
- System performance (to assess accuracy and precision).

D1.2 ENTRIX Internal Data Quality Control Procedures

ENTRIX has established an internal quality assurance program to ensure that all project analytical data are tracked within a COC database system and are of reliable and comparable data quality. The project quality assurance manager will be responsible for ensuring that ENTRIX internal quality control procedures are followed for all project analytical data.

The COC database system allows ENTRIX to track samples and their results to ensure that the project DQO for completeness is met. Samples and data are tracked in a COC database system by their COC number. The COC number along with the date the laboratory received the samples for analyses are entered into the COC database system from the information on the experimental copy of the COC. When the final laboratory reports are completed, the laboratory report number along with the date and initials of the ENTRIX personnel who have reviewed the report is entered into the COC database system according to the COC number.

A limited internal data validation is performed on all project analytical data when the final report is reviewed by ENTRIX. The limited data validation will include a data completeness review of each data package, and a limited review of QA/QC parameters as indicated in the national functional guidelines to ensure that all project analytical data are of reliable and comparable data quality. Specifically, the following QA/QC parameters will be reviewed:

- Holding times (to assess potential for degradation that will affect accuracy)
- Blanks (to assess contamination for all compounds)
- MS/MSD or laboratory control spike/spike duplicates (to assess accuracy of the methods and precision of the method relative to the specific sample matrix)

- Internal standards (to assess method accuracy and sensitivity)
- Compound reporting limit and MDL (to assess sensitivity as compared to project-specific requirements); and
- Experimental duplicate RPDs (to assess precision of the method relative to experimental sampling techniques, the specific sample matrix, and representativeness of the sample aliquot to the treatment sampled).

The results of this limited data validation and any corrective actions implemented are recorded on a QA/QC worksheet. The data reviewer will initial and date the QA/QC worksheet. The project manager will provide secondary review of the QA/QC worksheet and will also initial and date the QA/QC worksheet. The initialed and dated QA/QC worksheet will be attached to the final analytical laboratory report that is retained in the project files.

D2 VERIFICATION AND VALIDATION METHODS

The data validation process is conducted to assess the effect of the overall sampling and analysis process on the usability of the data. There are two areas of review: laboratory performance evaluation and the effect of matrix interferences. Evaluation of laboratory performance is a check for compliance with the method requirements and is a straightforward examination. The laboratory either did or did not analyze the samples within the quality control limits of the analytical method and according to protocol requirements. The assessment of potential matrix effects consists of a quality control evaluation of the analytical results and also the results of testing blank, duplicate, and matrix spike samples, and then assessing how, if at all, the matrix effect will affect the usability of the data.

All analytical data will be supported by a data package. The data package contains the supporting quality control data for the associated samples. The data validation report deliverables will include the following information:

- A comprehensive narrative detailing all quality control exceedances, explaining qualifications of data results. In cases where data are qualified due to quantifiable quality control exceedances, the bias (high or low) will be identified;

- Data summary tables in Microsoft® Excel format reporting all data results with the qualifiers that were added during the data validation review. These tables will include sample ID, laboratory ID, date sampled, sample type (e.g., experimental duplicate, experimental blank), units, concentration of analytes, and validation qualifiers. These tables may be modified to report other information as needed (such as depth of soil samples, date analyzed, dilution factor)
- Resubmittal requests sent to the laboratory indicating missing information, verification of analytical information, etc.
- EDDs compatible with the project database. These electronic deliverables will contain the validated results and qualifications as presented in the data summary tables of the validation reports. In addition, the validation reports can be submitted in electronic format for inclusion in interim remedial investigation data deliverables.

Before the laboratory releases each data package, the laboratory must carefully review the sample and laboratory performance quality control data to verify sample identity and also the completeness and accuracy of the sample and quality control data. This is performed through three levels of laboratory data review starting with 100 percent verification performed by the laboratory analyst, followed by a second-level review performed by a peer, supervisor, or designee. The laboratory project manager performs the third and final laboratory review to assure that project requirements are met for the analyses performed.

Data validation is at times based on best professional judgment. In order to achieve consistent data validation, data worksheets will be completed for each data validation effort. A data review worksheet is a summary form on which the data validator records data validation notes and conclusions specific to each analytical method. The worksheets will help the validator to track and summarize the overall quality of the data. Sample results will then be qualified as appropriate, following EPA protocols. Samples that do not meet the acceptance limit criteria will be indicated with a qualifying flag, which is a one or two-letter abbreviation that indicates a problem with the data (Table D2-1).

The data verification process begins once the data packages for each project have been validated. During verification, the entire data set will be verified for overall trends in data quality and usability. Information summarized as part of the data quality verification will include frequencies of detection, dilution factors that might affect data

usability, and patterns of target compound distribution. The data set also will be evaluated to identify potential data limitations or uncertainties in the laboratory. The trend analysis results will be included in the validation summary report, which will be submitted to the project manager at the end of the study efforts. The validation report and notes will be archived with the analytical data.

D3 RECONCILIATION WITH USER REQUIREMENTS

An assessment of the usability of the validated data compared to the data validation criteria and DQOs will be provided. The usability assessment will be performed in accordance with USEPA (1992) and best professional judgment. The data auditor will delineate major and minor deficiencies in the data, their effects on the reported results, and determination of usability for each compound reported in each sample included in the data package. The usability assessment will provide an overall summary of data quality. It defines acceptability or problems with accuracy, precision, sensitivity, and representativeness of the results with clear guidance to the data users of the uncertainties in the data that have been qualified as estimated (J) and a quantification of these uncertainties (e.g., bias high by a maximum of 80 percent), wherever possible. The data auditor may determine specific results to be unusable because of cumulative effects of quality control exceedances (i.e., an "R" qualifier will be applied to the result). Alternatively, based upon the EPA guidelines and best professional judgment, the data auditor may determine specific results to be usable for DQOs when they are not significantly outside the quality control criteria.

The final activity of the data validation process is to assess whether the data meet the DQOs. The final results, as adjusted for the findings of any data validation/data evaluation, will be checked against the DQOs and an assessment will be made as to whether the data are of sufficient quality to support the DQOs. The decision as to data sufficiency may be affected by the overall precision, accuracy, and completeness of the data as demonstrated by the data validation process. If the data are sufficient to achieve project objectives, the project manager will release the data and work can proceed. If the data are insufficient, corrective action will be required.

SECTION E: REFERENCES

ASTM. 2005. Standard guide for conducting early life-stage toxicity tests with fishes. ASTM E 1241–05. American Society for Testing and Materials, West Conshohocken, PA, USA.

ASTM. 2007. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM E729 – 96. Developed by Subcommittee: E47.01 |Book of Standards Volume: 11.06. American Society for Testing and Materials, Philadelphia, PA, USA.

ASTM. 2008. Standard Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians. ASTM E1192 – 97. American Society for Testing and Materials.

Ankley, G.T., W.J. Berry, D.M. Di Toro, D.J. Hansen, R.A. Hoke, D.R. Mount, M.C. Reiley, R.C. Swartz, and C.S. Zarba. 1996. Use of equilibrium partitioning to establish sediment quality criteria for nonionic chemicals: A reply to Iannuzzi et al. *Environ. Toxicol. Chem.* 15(7):1019-1014.

Antcliffe, B.L., Kieser, D., Lawrence, G., Lockhart, W.L., Metner, D.A. and Thompson, J.A.J., 1997. Monitoring of mountain whitefish, *Prosopium williamsoni*, from the Columbia River system near Castlegar, British Columbia: Final assessment of fish health and contaminants, July 1996. Canadian Technical Report of Fisheries and Aquatic Sciences 2184: xiii+79.

APHA. 1998. *Standard methods for the analysis of water and wastewater*. 20th Edition. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, DC. 1268 pp.

Besser, J.M., C.A. Mebane, D.R. Mount, C.D. Ivey, J.L. Kunz, I..E. Greer, T.W. May, and C.G. Ingersoll. 2007. Sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Onchorhynchus mykiss*) to acute and chronic toxicity of cadmium, copper and zinc. *Environ. Toxicol. Chem.* 26: 1657-1665.

Boyle, D.E., B.A. Bravender, T.J. Brown, D. Keiser, C.D. Levings, W.L. Lockhart, J.A. Servizi, and T.R. Whitehouse 1992. Baseline monitoring of mountain whitefish, *Prosopium williamsoni*, from the Columbia River system near Castlegar, British Columbia: Health, contaminants and biology. Canadian Technical Report of Fisheries and Aquatic Sciences, 1883: 64 pp. + appendices.

Bruggeman, W.A., A. Operhuizen, A. Wibenga, and O. Hutzinger. 1984. Bioaccumulation of super-lipophilic chemicals in fish. *Toxicol. Environ. Chem*. 7:173-189.

Bruno, J. 2004. Effects of two industrial effluents on juvenile white sturgeon (*Acipenser transmontanus*). Prepared for the Sturgeon Contaminants Working Group.

Clark, K.E., F.A.P.C. Gobas, and D. Mackay. 1990. Model of organic chemical uptake and clearance by fish from food and water. *Environ. Sci. Technol.* 24: 1203-1213.

Di Toro, D.M., C.S. Zarba, D.J. Hansen, W.J. Berry, R.C. Swartz, C.E. Cowan, S.P. Pavlou, H.E. Allen, N.A. Thomas, and P.R. Paquin. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals by using equilibrium partitioning. *Environ. Toxicol. Chem.* 10:1541-1583.

Di Toro, D.M., H.E. Allen, H. Bergman, J.S. Meyer, P.R. Paquin, and C.S. Santore. 2001. Biotic ligand model of the acute toxicity of metals. I. Technical basis. *Environ. Toxicol. Chem.* 20: 2383-2396.

Di Toro, D.M., J.A. McGrath, D.J. Hansen, W.J. Berry, P.R. Paquin, M. Rooni, K. Wu, and R.C. Santote. 2005. Predicting sediment metal toxicity using a sediment biotic ligand model: methodology and initial application. *Environ. Toxicol. Chem*. 24(10): 2410-2427.

Dwyer, F.J., F.L. Mayer, L.C. Sappington, D.R. Buckler, C.M. Bridges, I.E. Greer, D.K. Hardesty, C.E. Henke, C.G. Ingersoll, J.L. Kunz, D.W. Whites, T. Augspurger, D.R. Mount, K. Hattala, and G.N. Neuderfer. 2005. Assessing contaminant sensitivity of endangered and threatened aquatic species: Part I. Acute toxicity of five chemicals. *Arch. Environ. Contam. Toxicol.* 48:143-154.

Fisk, A.T., R.J. Norstrom, C.D. Cymbalisty, and D.C.G. Muir. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ. Toxicol. Chem.* 17(5):951-961.

Gobas, F.A.P.C., and D. Mackay 1987. Dynamics of hydrophobic organic chemical bioconcentration in fish. *Environ. Toxicol. Chem.* 6:495-504.

Gobas, F.A.P.C., A. Opperhuizen, and O. Hutzinger 1986. Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation. *Environ. Toxicol. Chem.* 5(7):637-646.

Golder Associates Ltd. 2007. White sturgeon spawning at Waneta, 2007 investigations. Report prepared for Teck Cominco Metals Ltd. Trail Operations. Golder Report No. 07-1480-0031F: 28p.

Grosell, M., C. Nielsen, and A. Bianchini. 2002. Sodium turnover rate determines sensitivity to acute Cu and silver exposure in freshwater animals. *Comp. Biochem. Physiol.* C 133: 287-303.

Grosell, M., J. Blancharda, K.V. Brixb, and R. Gerdes. 2007. Physiology is pivotal for interactions between salinity and acute Cu toxicity to fish and invertebrates. *Aquat. Toxicol.* 84: 162–172.

Haddy, J.A., and N.W. Pankhurst. 1999. Stress-induced changes in concentrations of plasma sex steroids in black bream. *J. Fish Biol.* 55:1304–1316.

Hildebrand, L., C. McLeod, and S. McKenzie. 1999. Status and management of white sturgeon in the Columbia River in British Columbia, Canada: an overview. *J. Appl. Ichthyol.* 15: 164-172.

Hinck, J.E., C.J. Schmitt, V.B. Blazer, N.D. Denslow, T.M. Bartish, P.J. Anderson, J.J. Coyle, G.M. Dethoff, and D.E. Tillitt. 2006. Environmental contaminants and biomarker responses in fish from the Columbia River and its tributaries: Spatial and temporal trends. *Sci. Total Environ.* 366: 549-578.

Holcombe, G.W., D.A. Benoit, E.N. Leonard, and J.M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Board Can.* 33:1731-1741.

Holcombe, G.W., D.A. Benoit, and E.N. Leonard. 1979. Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*). *Trans. Am. Fish. Soc.* 108:76-87.

Howell, M.D., and J.G. McLellan. 2006. Lake Roosevelt white sturgeon recovery project, annual report January 2004 – March 2005. Bonneville Power Administration, Project No. 1995-027-00. Portland, OR.

HydroQual Inc. 2005. The Biotic Ligand Model Windows Interface, Version 2.2.3: User's Guide and Reference Manual. Mahway, NJ.

Johnson, A., and D. Serdar 1991. Metals concentrations in Lake Roosevelt (Columbia River) largescale suckers. Internal memo to *C. Nuechtherlein*, Washington State Department of Ecology, Olympia. Olympia, WA.

Jonker, M.T.O., and S.A. van der Heijden 2007. Bioconcentration factor hydrophobicity cutoff: An artificial phenomenon reconstructed. *Environ. Sci. Technol.* 41(21):7363-7369.

Kent, M.L., V. Watral, C. Whipps, M.E. Cunningham, C.D. Criscione, J.R. Heidel, L.R. Curtis, J. Spitsbergen, and D.E. Markle. 2004. A digenean metacercaria (*Apophallus* sp.) and a myxozoan (*Myxobolus* sp.) associated with vertebral deformities in cyprinid fishes from the Willamette River, Oregon. *J. Aquat. Anim. Health* 16(3):116-129.

Kruse, G.O., and D.L. Scarneccia. 2002a. Assessment of bioaccumulated metal and organochlorine compounds in relation to physiological biomarkers in Kootenai River white sturgeon. *J. Appl. Ichthyol.* 18: 430–438

Kruse, G.O., and D.L. Scarneccia. 2002b. Contaminant uptake and survival of white sturgeon. American Fisheries Society Symposium 28: 151-160.

Kruse, G., and M. Webb. 2006. Upper Columbia River white sturgeon contaminant and deformity evaluation survey. Prepared for the Upper Columbia River White Sturgeon Recovery Team Contaminants Sub-Committee.

Kubokawa K., T. Watanabe, M. Yoshioka, and M. Iwata. 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. *Aquaculture* 172:335-349.

Lapirova T.B., V.R. Mikryakova, A.S. Mavrin, and G.A. Vinogradova. 2000. Effect of sublethal concentrations of mercury, cadmium, and copper salts on the lysozyme content in fry of the Lena River Sturgeon *Acipenser baeri*. *J. Evol. Biochem. Physiol.* 36:47-51.

Law, M. 2001. Differential diagnosis of ulcerative lesions in fish. *Environ. Health Perspect.* 109: 681-686.

McKim, J.M., and D.A. Benoit. 1971. Effects of long-term exposures to copper on the survival, growth, and reproduction of brook trout. *J. Fish. Res. Board Can.* 28:655-662.

Mebane, C.A., D.P. Hennessy, and F.S. Dillon. 2008. Developing acute-to-chronic toxicity ratios for lead, cadmium, and zinc using rainbow trout, a mayfly, and a midge. *Water Air Soil Pollut*. 188:41–66.

Nener, J., D. Kieser, J.A. Thompson, W.L. Lockhart, D.A. Metner, and R. Roome. 1995. Monitoring of mountain whitefish, *Prosopium williamsoni*, from the Columbia River System near Castlegar, British Columbia: Health parameters and contaminants in 1992. *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2036:89. Vancouver, BC, Canada, Department of Fisheries and Oceans.

Paquin, P.R., R.C. Santore, K.B. Wu, P.J. Anid, P.D. Kavvadas, and D.M. Di Toro. 2000. Revisiting the aquatic impacts of copper discharged by water-cooled copper alloy condensers used by power and desalination plants. *Environ. Sci. Policy* 3: S165-S174.

Paulson, A.J., R.J. Wagner, R.F. Sanzolone, and S.E. Cox. 2006. Concentrations of elements in sediments and selective fractions of sediments, and in natural waters in contact with sediments from Lake Roosevelt, Washington, September 2004, U.S. Geological Survey: 94.

Paulson, A.J., and S.E. Cox. 2007. Release of elements to natural water from sediments of Lake Roosevelt, Washington, USA. *Environ. Toxicol. Chem.* 12:2550-2559.

Posthuma, L., G.W. Suter II, and T.P. Traas 2002. Species sensitivity distributions in ecotoxicology, Lewis Publishers, Boca Raton, FL.

R.L. & L. Environmental Services Ltd. 1994. Status of white sturgeon in the Columbia River, B.C. Report prepared for BC Hydro, Environmental Affairs, Vancouver, B.C. by R.L. & L. Environmental Services Ltd., Vancouver, B.C. Report #377F 101 p + 5 app.

R.L. & L. Environmental Services Ltd. 1995. White Sturgeon in the Columbia River, B.C., 1994 study results. Report prepared for BC Hydro, Environmental Affairs, Vancouver, B.C. R.L. & L. Report No. 377D: 77 p. + 4app.

R.L. & L. Environmental Services Ltd. 1996. Columbia River white sturgeon investigations, 1995 study results. Report prepared for BC Hydro, Kootenay Generation, Vancouver, B.C. and B.C. Ministry of Environment, Lands and Parks, Nelson Region. R.L. & L. Report No. 96-377F: 94 p. + 6 app.

Spokane Tribe of Indians. 2003. Surface water quality standards, Resolution 2003-259, Spokane Tribe of Indians.

Suter, G.W., D.S. Vaughn, and R.H. Gardner. 1983. Risk assessment by analysis of extrapolation error: a demonstration for effects of pollutants on fish. *Environ. Toxicol. Chem.* 2: 369-378.

TAI. 2009a. Upper Columbia River: Work plan for the remedial investigation and feasibility study. Teck American Incorporated, Spokane, WA. As modified by U.S. Environmental Protection Agency.

TAI. 2009b. Upper Columbia River: Draft baseline ecological risk assessment work plan. Prepared for Teck American Incorporated, Spokane, WA. Prepared by Parametrix, Inc., Integral Inc., and HydroQual, Inc. (March 2009).

TCAI. 2008. Upper Columbia River, screening level ecological risk assessment. Prepared by Parametrix, Inc., Bellevue, WA, and Integral Consulting Inc., Mercer Island, WA. Teck Cominco American, Incorporated, Spokane, WA.

UCWSRI. 2002. Upper Columbia White Sturgeon Recovery Plan. Upper Columbia White Sturgeon Recovery Initiative. http://uppercolumbiasturgeon.org/RecoveryEfforts/Recovery.html. November 28.

USEPA. 1984. Guidelines for deriving numerical aquatic site-specific water quality criteria by modifying national criteria. EPA/600/3-84/099. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA. 1992. Guidance for data usability in risk assessment (Part A), EPA Publication 9285.7-09A, U.S. Environmental Protection Agency, April 1992.

USEPA. 1994. Use of the water effect ratio in water quality standards. EPA-823-B-94-001. Office of Science and Technology, Washington, DC. 153 pp.

USEPA. 1996. Sampling ambient water for trace metals at EPA water quality criteria levels. EPA-821-R-96-011. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 1999. 1999 update of ambient water quality criteria for ammonia. EPA 822-R-99-014. Office of Water, Washington, DC.

USEPA. 2001. 2001 Update of Ambient Water Quality Criteria for Cadmium (EPA-822-R01-001). Washington, DC, Office of Water, U.S. Environmental Protection Agency. 35 pp.

USEPA. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5th Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 2004. USEPA contract laboratory program national functional guidelines for inorganic data review. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response, Washington, DC.

USEPA. 2005a. "ECOTOX Database." Obtainable on the Internet at http://www.epa.gov/ecotox/. U.S. Environmental Protection Agency.

USEPA. 2005b. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: Metal mixtures (cadmium, copper, lead, nickel, silver, and zinc), U.S. Environmental Protection Agency, Office of Water, Washington, DC. 121 pp.

USEPA. 2006a. Guidance on systematic planning using the data quality objectives process (EPA QA/G-4). EPA/240/B-06/001. U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2006b. National recommended water quality criteria. http://www.epa.gov/waterscience/criteria/wqctable/index.html. U.S. Environmental Protection Agency.

USEPA. 2006c. Settlement agreement for implementation of remedial investigation and feasibility study at the Upper Columbia River Site. June 2, 2006. U.S. Environmental Protection Agency, Region 10, Seattle, WA.

USEPA. 2007. Aquatic life ambient freshwater quality criteria – copper. (EPA-822-R-07-001). U.S. Environmental Protection Agency, Office of Water, Washington, DC. 39 pp.

USEPA. 2008. Sediment assessment and monitoring sheet (SAMS) #1: Using fish tissue data to monitor remedy effectiveness. U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation and Office of Research and Development. OSWER Directive 9200.1-77D. July 2008.

USFWS and USGS. 2008. Summary of Kootenai River white sturgeon studies. information sheet. U.S. Fish and Wildlife Service, Upper Columbia Fish and Wildlife Office, Spokane, WA, USA, and U.S. Geological Survey (2007/2008).

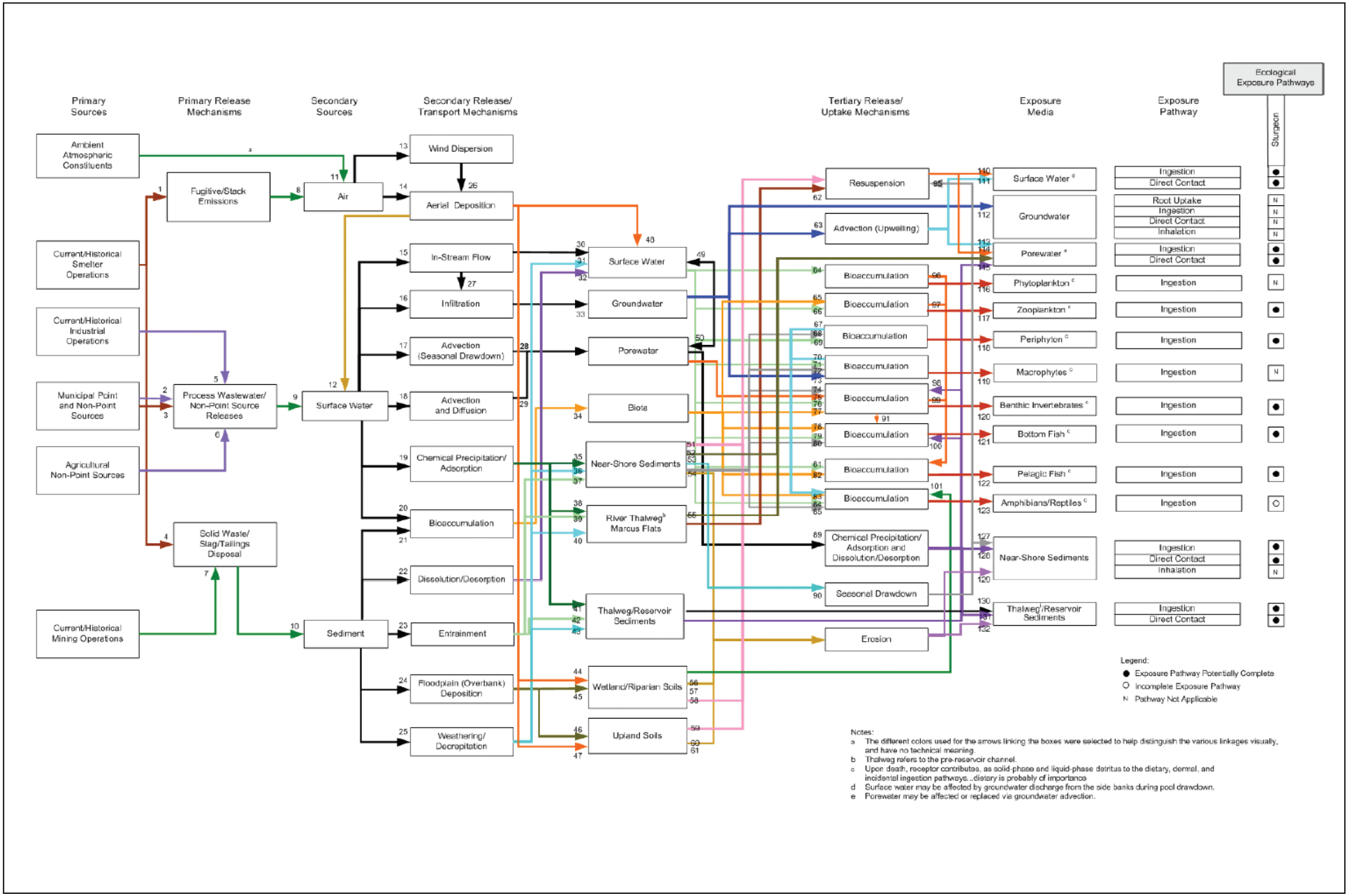


Figure A4-1. General Conceptual Site Model for White Sturgeon (and other benthic-feeding fish).
Source: Modified from TCAI (2008)

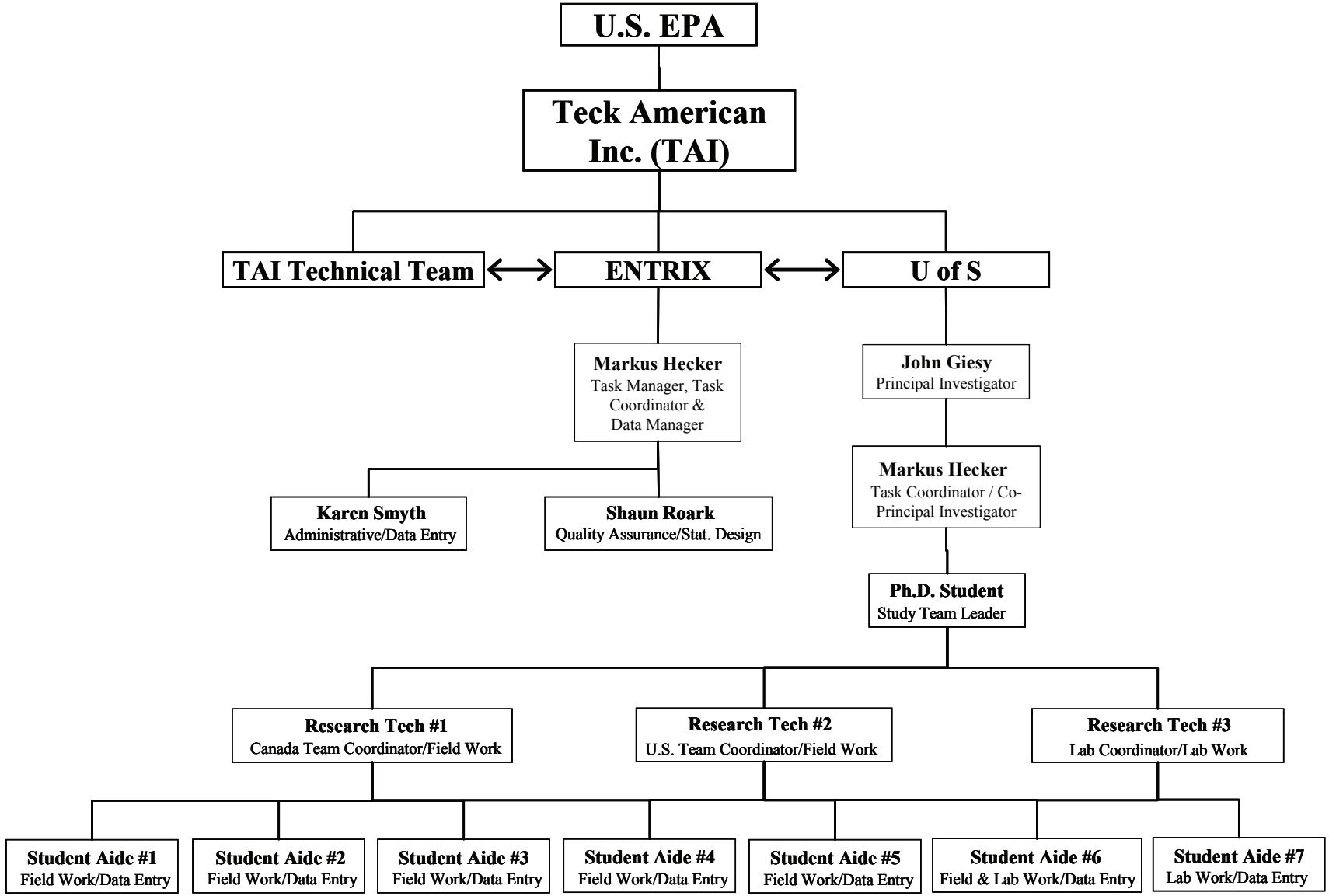
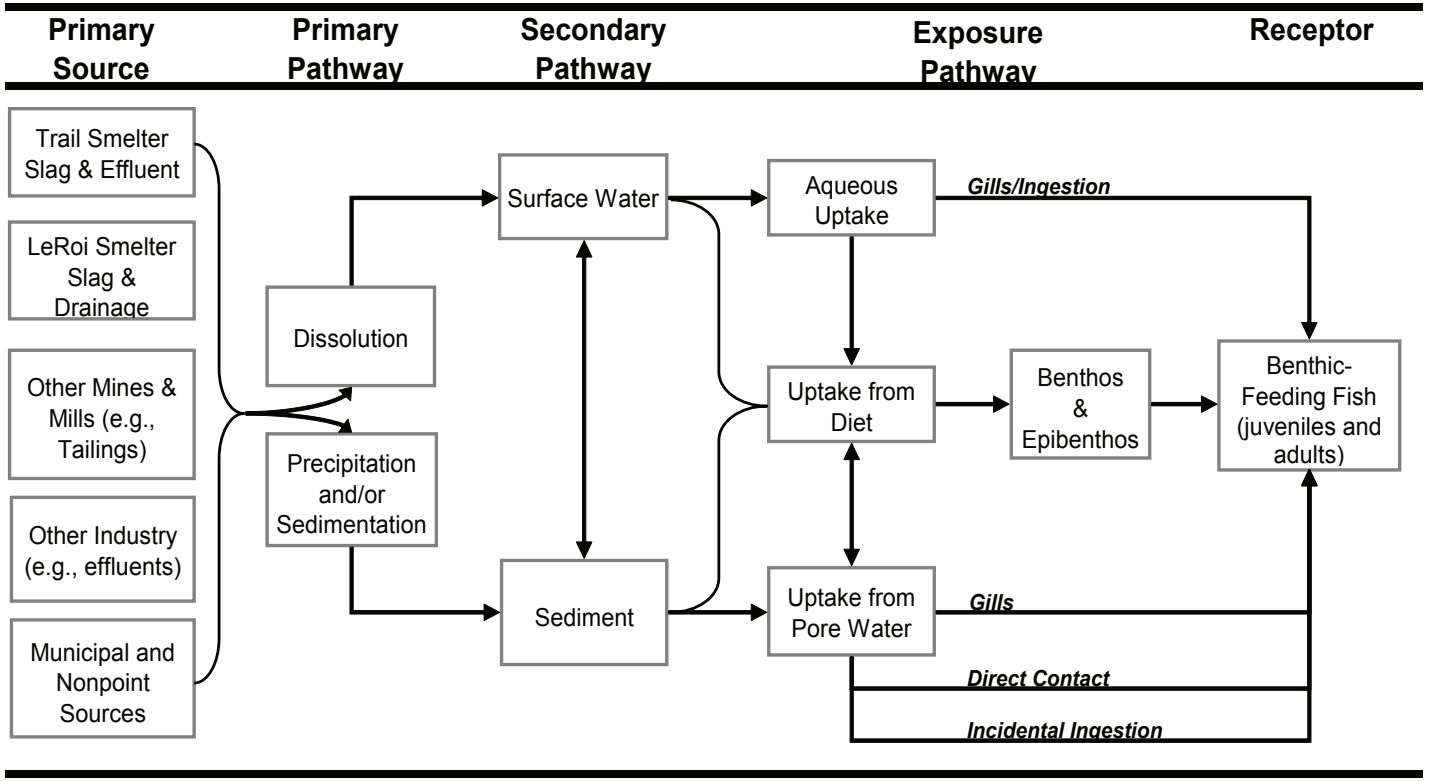


Figure A4-2. Task Organizational Chart.



Note: Sturgeon/benthic-feeding fish CSM has been simplified from the overall CSM. Please refer to overall CSM for detailed pathways/interactions.

Figure A4-3. Simplified Conceptual Site Model for White Sturgeon and Other Benthic-Feeding Fish Species in the UCR.

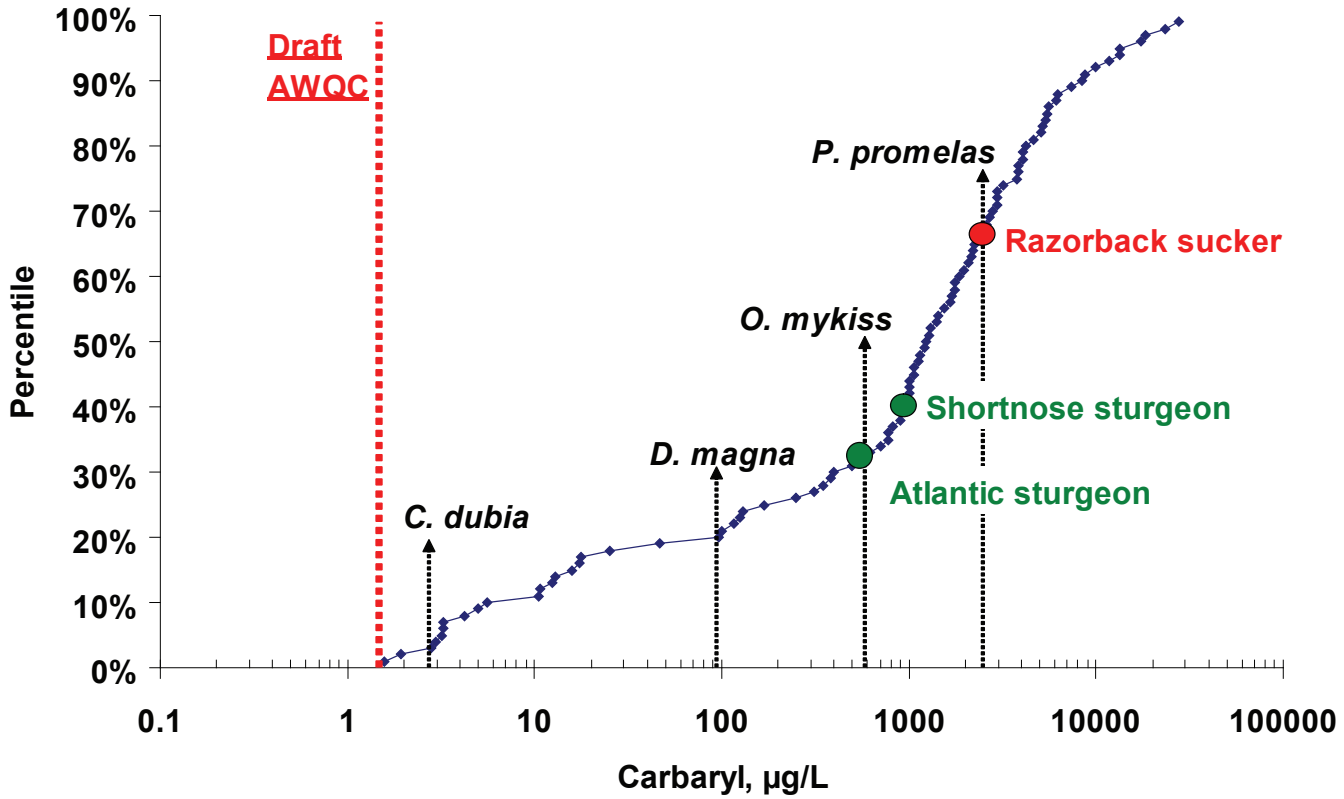


Figure A4-4. Relative Acute Sensitivities of Sturgeon, Sucker, and Other Species of Aquatic Life to the Carbamate Insecticide Carbaryl. **Note:** Acute LC50 values are divided by 2 for comparison to the chronic ambient water quality criterion (AWQC).

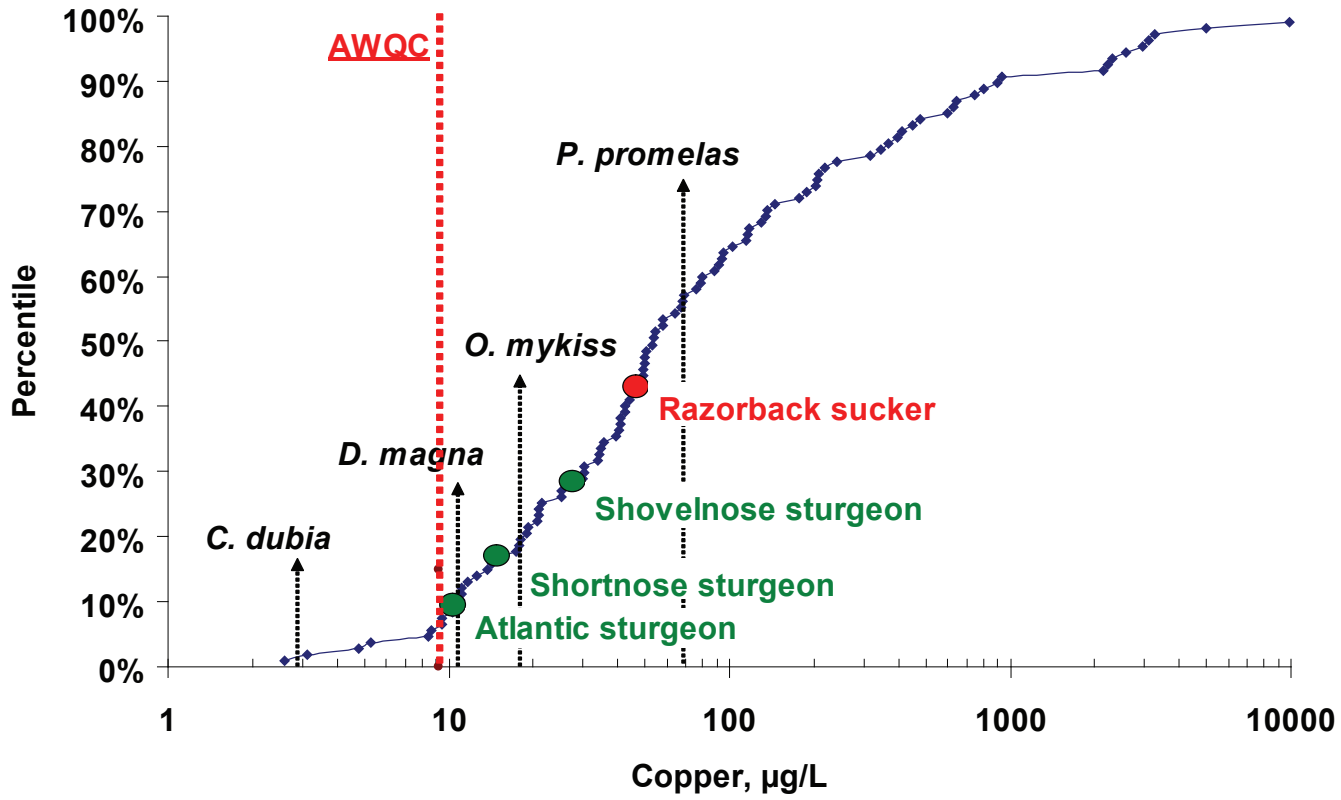


Figure A4-5. Relative Acute Sensitivities of Sturgeon, Sucker, and Other Species of Aquatic Life to Copper.
Note: Acute LC50 values are divided by 2 for comparison to the chronic ambient water quality criterion (AWQC) and normalized to 50 mg/L hardness.

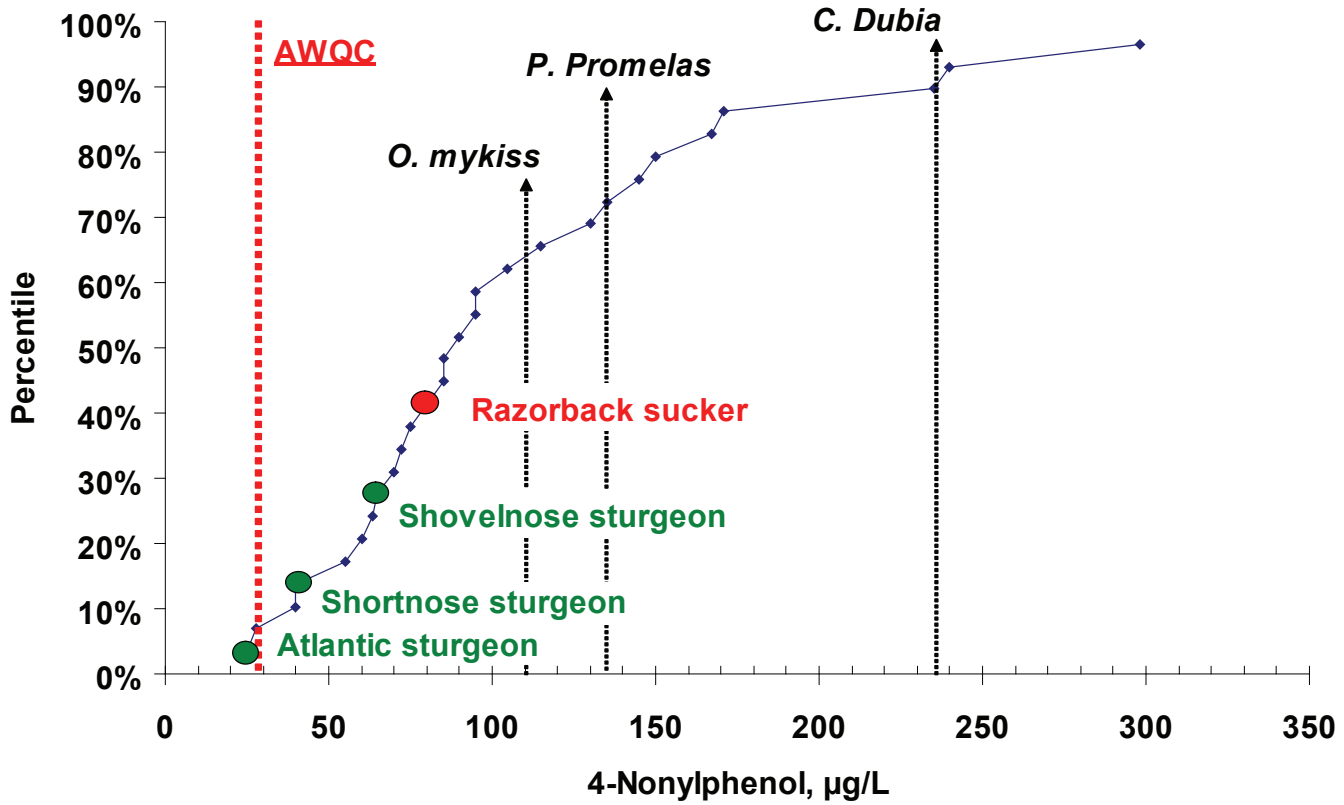


Figure A4-6. Relative Acute Sensitivities of Sturgeon, Sucker, and Other Species of Aquatic Life to the Surfactant 4-Nonylphenol. **Note:** Acute LC50 values are divided by 2 for comparison to the chronic ambient water quality criterion (AWQC).

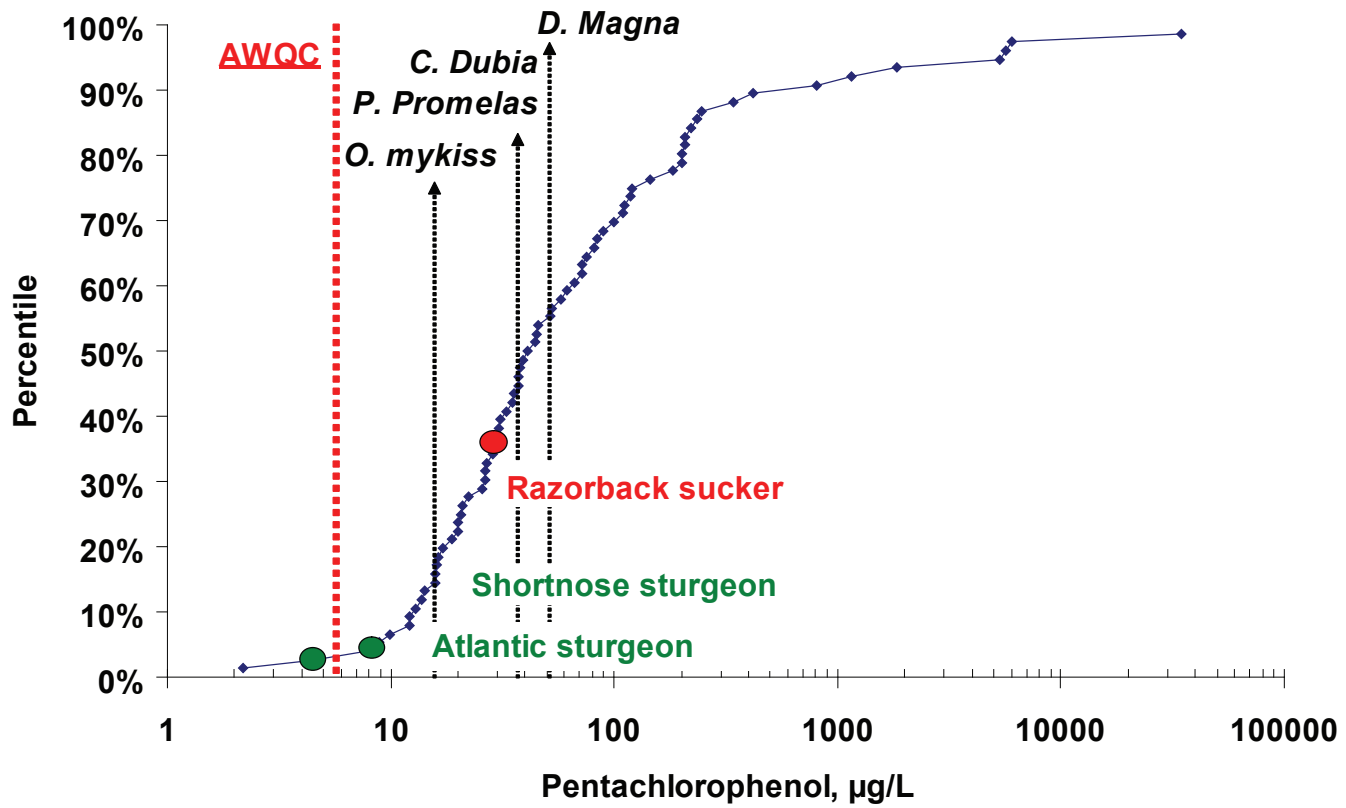


Figure A4-7. Relative Acute Sensitivities of Sturgeon, Sucker, and Other Species of Aquatic Life to Pentachlorophenol.
Note: Acute LC50 values are divided by 2 and normalized to pH 6.5 for comparison to the chronic ambient water quality criterion (AWQC).

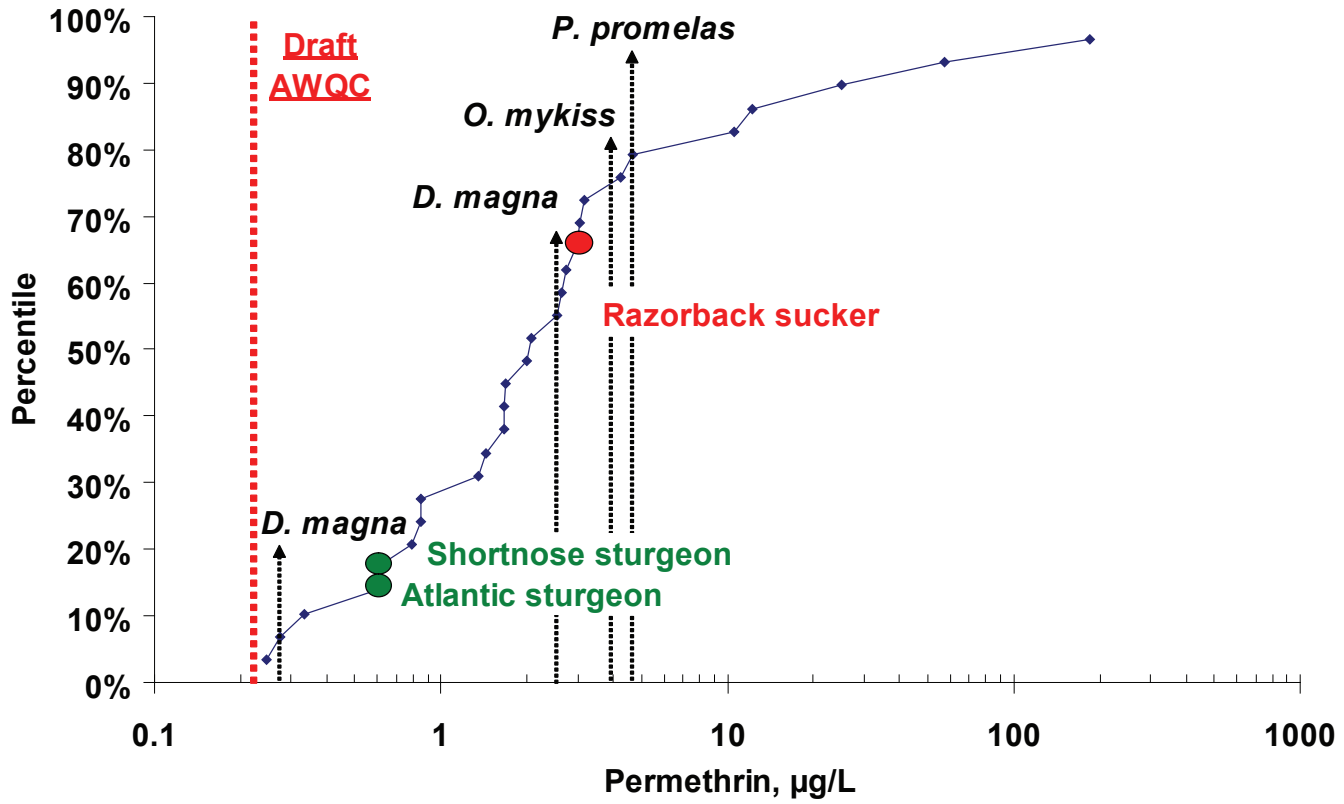


Figure A4-8. Relative Acute Sensitivities of Sturgeon, Sucker, and Other Species of Aquatic Life to the Insecticide Permethrin.
Note: LC50 values are divided by 2 for comparison to the draft chronic ambient water quality criterion (AWQC).

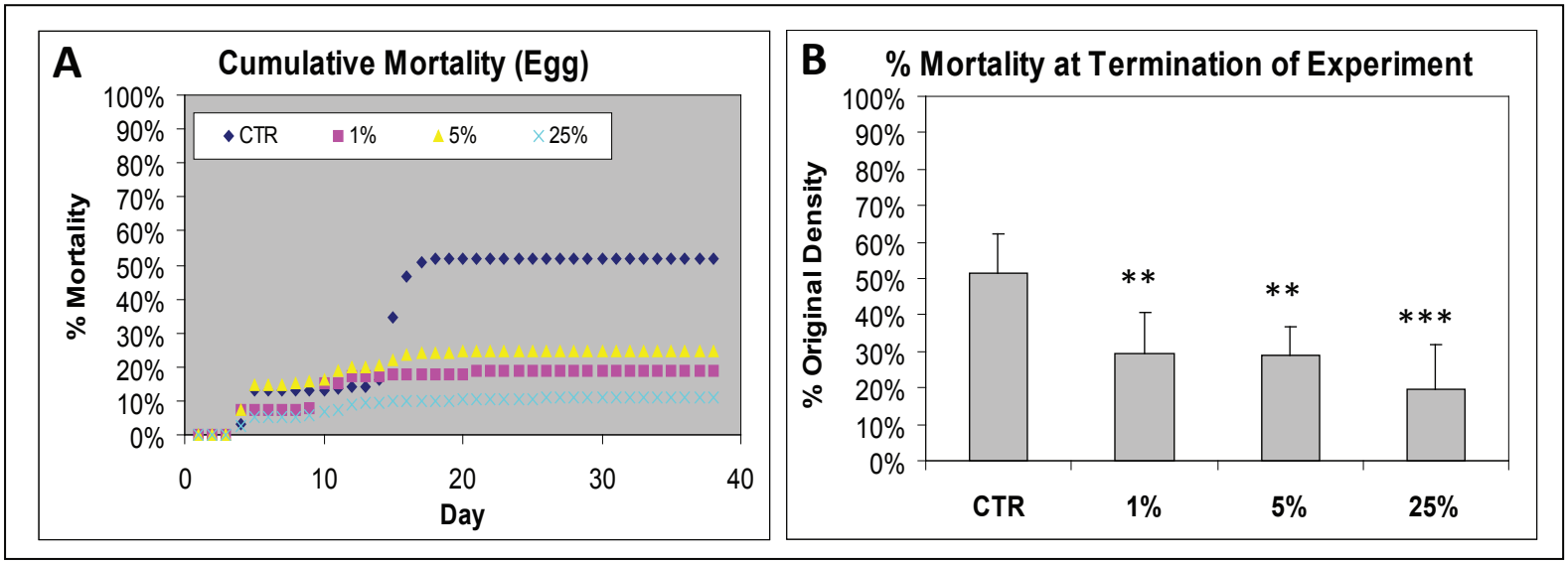


Figure A5-1. Cumulative Mortalities of White Sturgeon Larvae/Fry Exposed to 1, 5, and 25 Percent Effluent, and in the Controls over the Duration of the Experiment (A) and at Termination of the Studies (B).
Note: Asterisks indicate significant differences from the controls,
** $p<0.01$; *** $p<0.001$

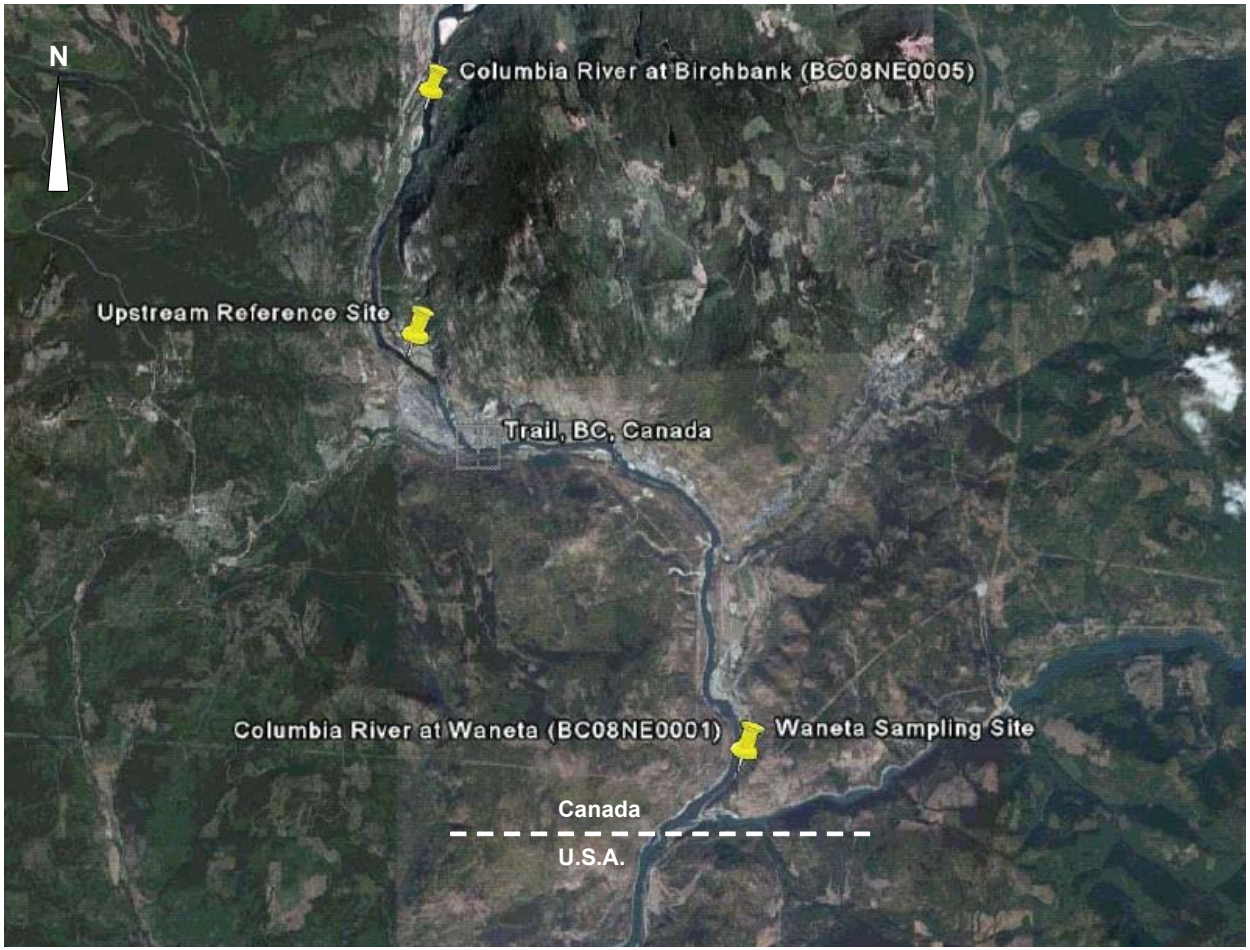


Figure A5-2. *In Situ* Study Sites and Federal-Provincial Surface Water Monitoring Stations (BC08NE0005 and BC08NE0001) Upstream and Downstream of Teck's Trail Facility in British Columbia, Canada.

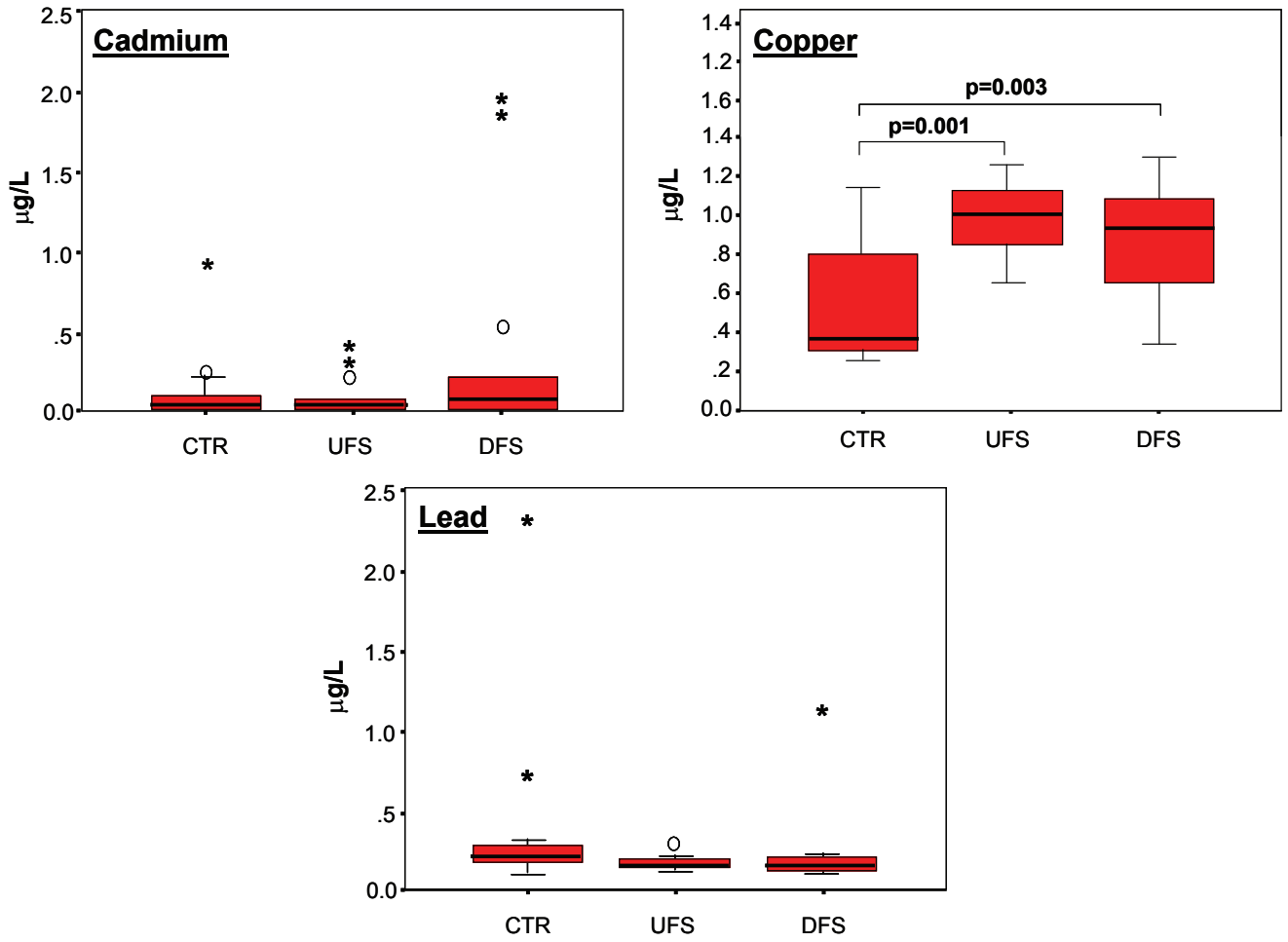


Figure A5-3. Tentative Concentrations of Cadmium, Copper, and Lead in the Filtered City Water Control (CTR) and in Columbia River Surface Water Up- (UFS) and Downstream (DFS) of Teck's Trail Facility during the Exposure Studies.

Note: Total number of samples: CTR=15; UFS=14; DFS=15. Data analysis was conducted using the BoxPlot Function of Systat 12 (Systat Software, Inc., Chicago, IL). Asterisk represents far outside values. Brackets indicate significant differences between treatment groups (Mann-Whitney U test).

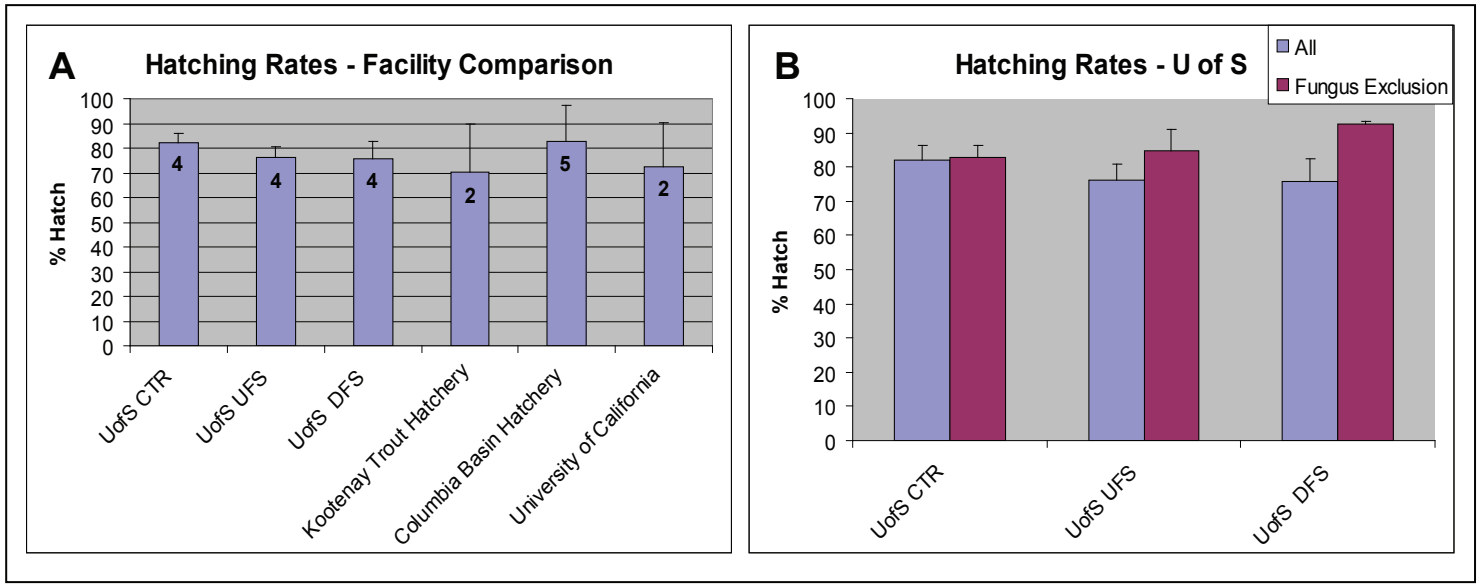


Figure A5-4. Hatch Rates of Fertilized White Sturgeon Eggs Observed during the 2008 Surface Water Toxicity Studies After and Before Exclusion of Eggs Removed Due to Fungus Infection (A) and Comparison of Data with Hatch Rates Reported by Three Other Institutions: Kootenay Trout Hatchery, Columbia Basin Hatchery and University of California (B).
Note: Error bars = 1 x SD. Numbers in bars represent number of replicates used to calculate average values.

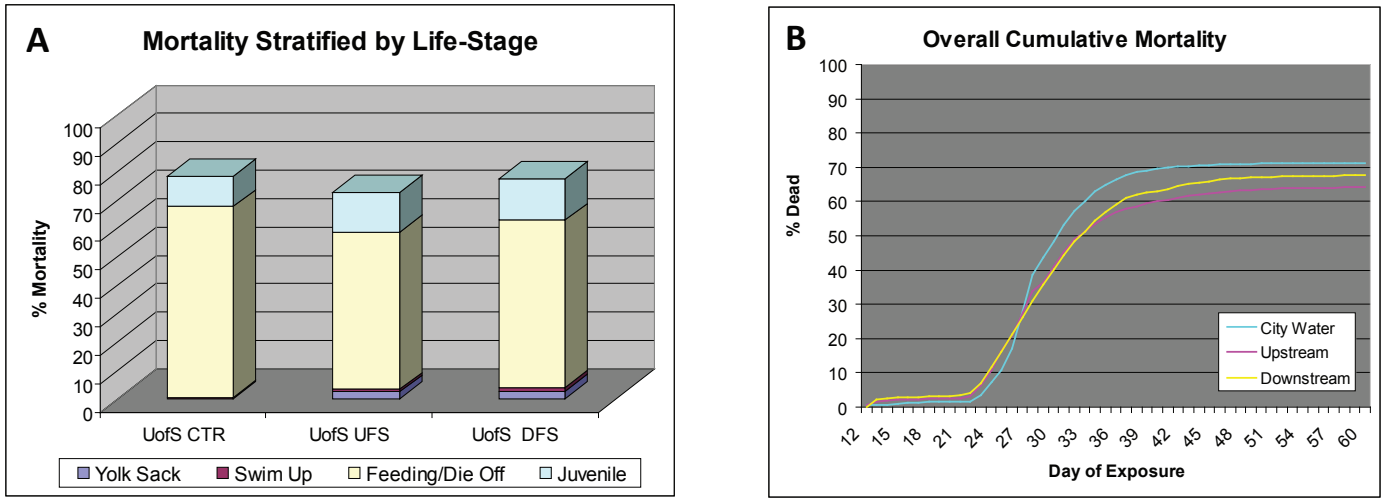


Figure A5-5. Mortality Rates of Sturgeon Fry/Juveniles in the Different Exposure Groups.
Note: A: Mortalities stratified by life stage. The different portions of the columns represent different life stages. B: Cumulative mortalities between hatch and termination of studies.

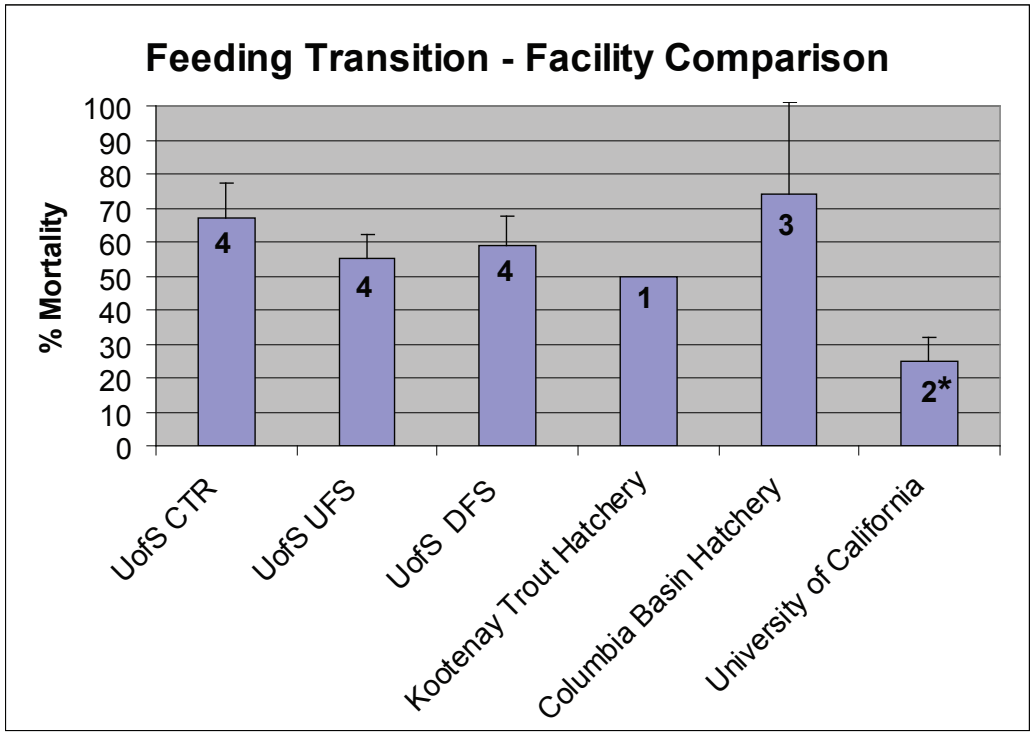


Figure A5-6. Mortality Rates of Early Life Stages of White Sturgeon Observed during the 2008 Surface Water Toxicity Studies and at Three Other Institutions: Kootenay Trout Hatchery, Columbia Basin Hatchery, and University of California.
Note: The University of California data represent "survival rates under optimum conditions" and are likely to underestimate average mortalities. Error bars = 1 x SD. Numbers in bars represent number of replicates used to calculate average values.

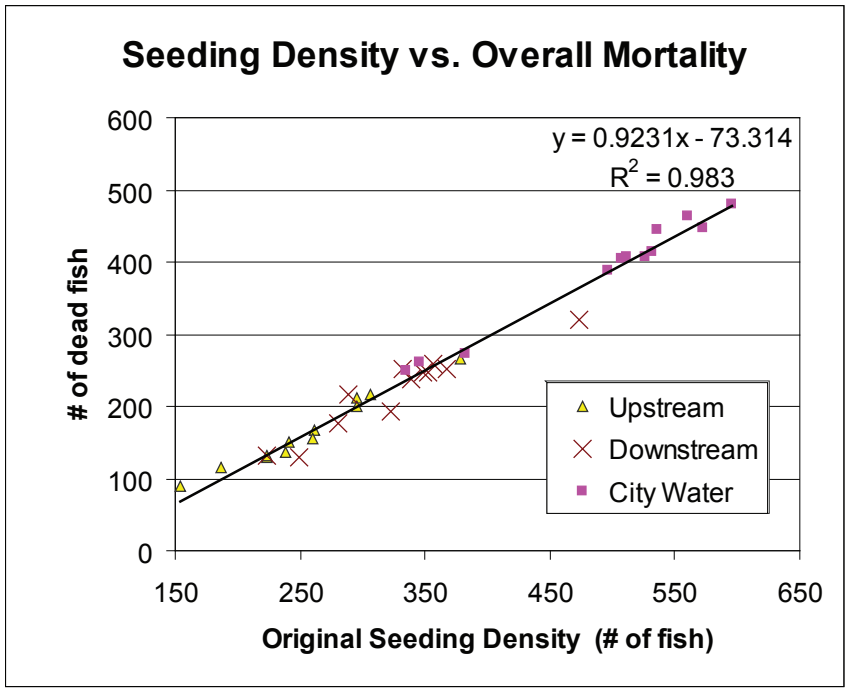


Figure A5-7. Linear Regression between Overall Number of Dead Fish and Original Seeding Densities of Fry.

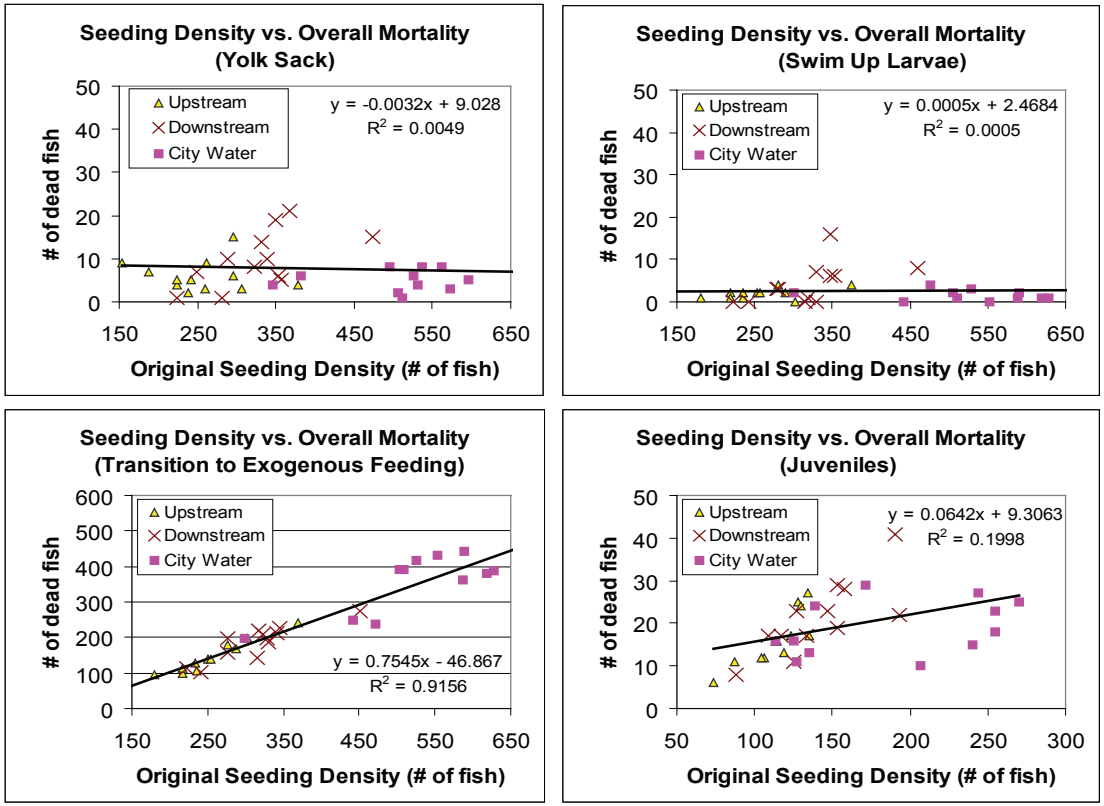
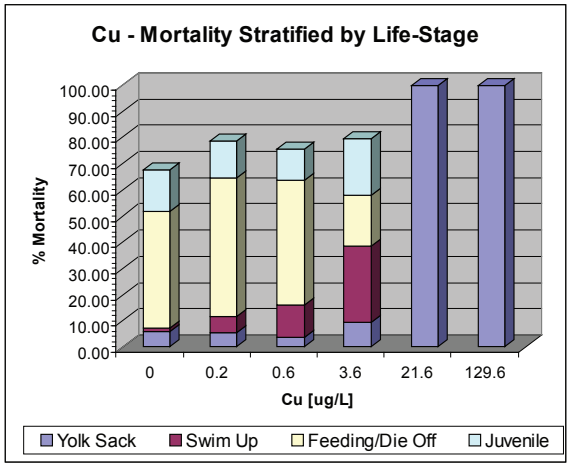
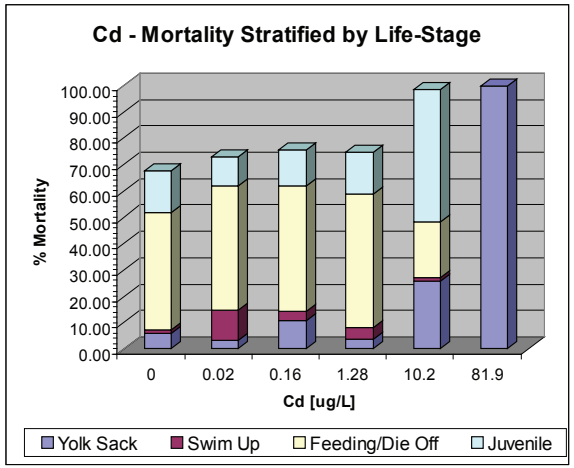


Figure A5-8. Linear Regressions between Overall Number of Dead Fish.





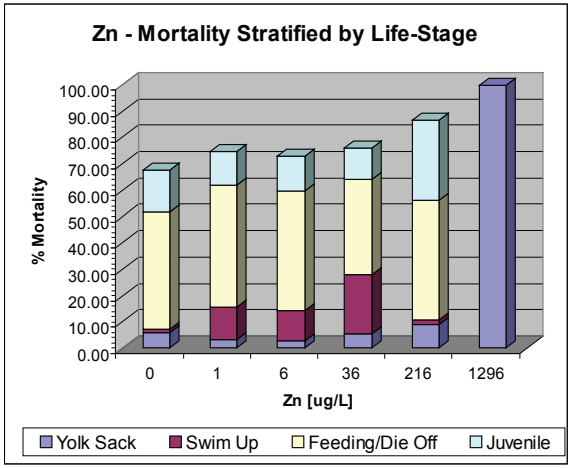


Figure A5-9. Mortality of Early Life Stages of White Sturgeon after Exposure to Copper, Cadmium, and Zinc from 8 Hours post Fertilization through ~60 Days post Hatch under Flow-Through Conditions in Laboratory Water at U of S. **Note:** Data points represent the mean out of four (controls) and two (metal exposure groups) replicate measurements. *** = significantly different from controls (=0) at $p < 0.001$ (2-sided Dunnett's test).

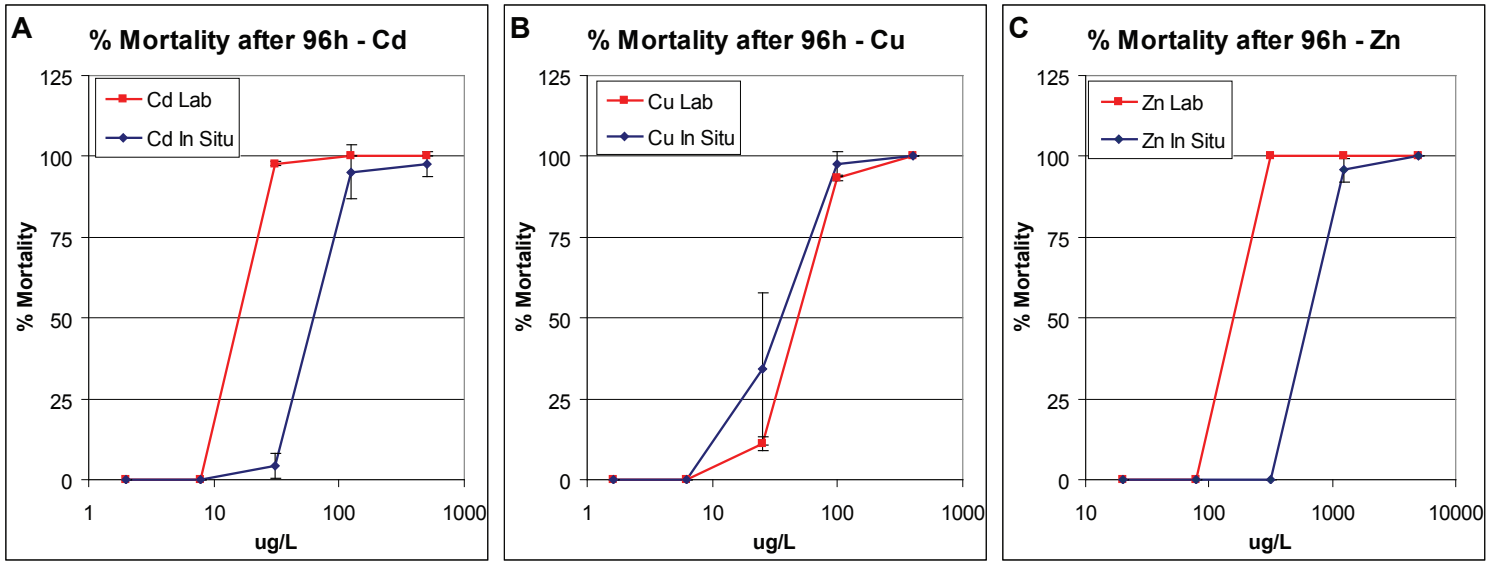
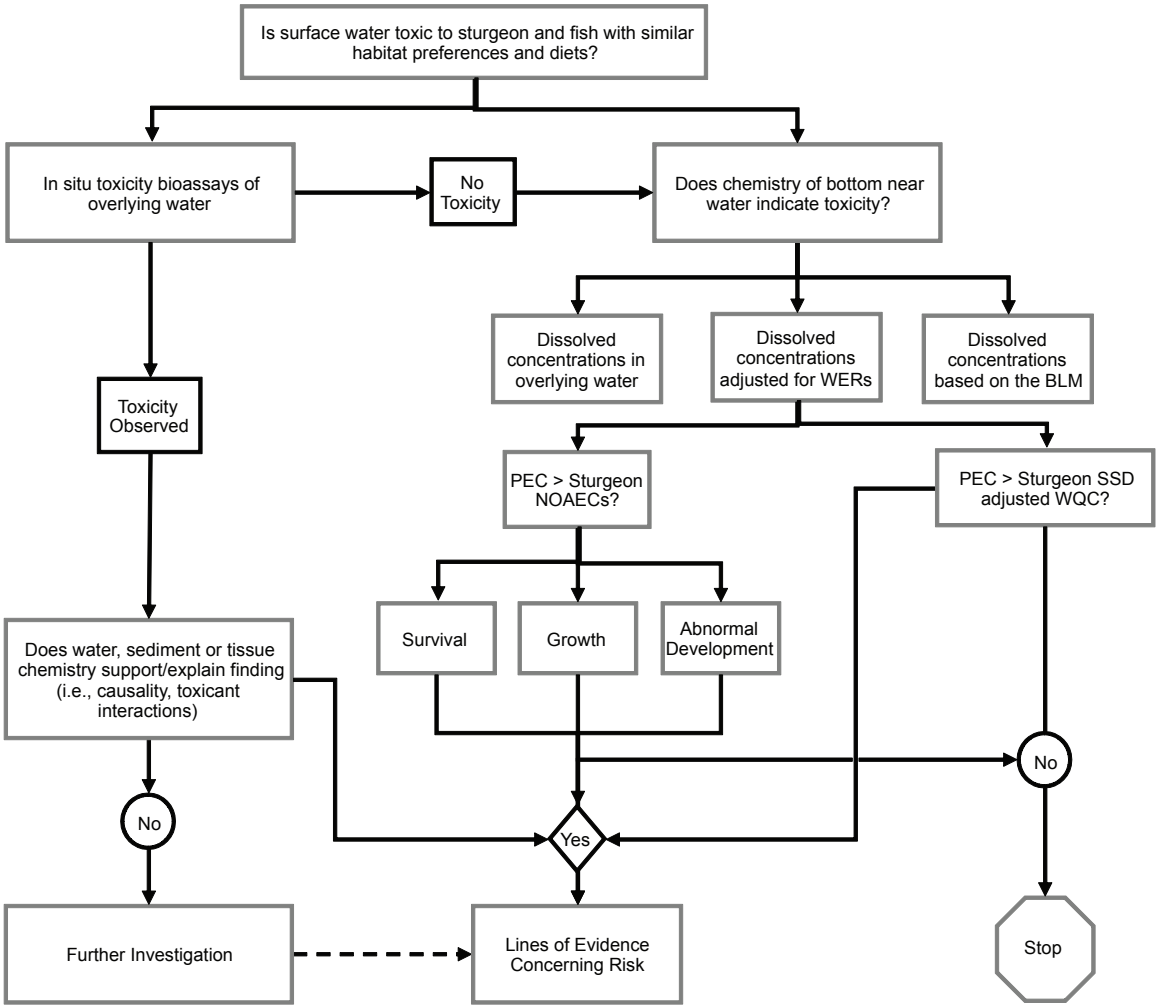
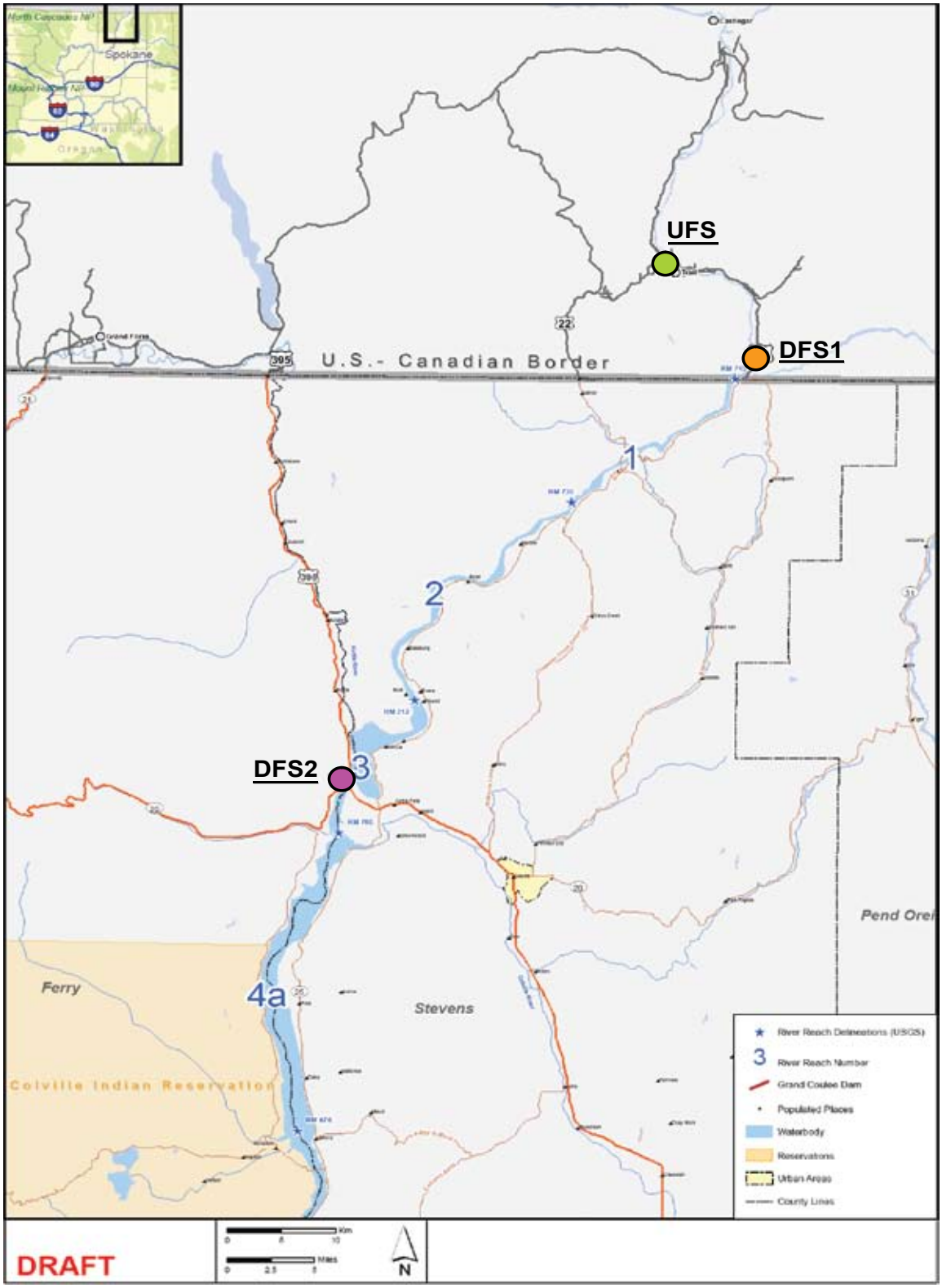


Figure A5-10. Mortality of White Sturgeon Fry (~8-10 Days post Hatch) after Exposure to Cadmium (A), Copper (B), and Zinc (C) for 96 Hours under Static Renewal Conditions in Columbia River Water in the Field (*In Situ*) and Laboratory Water at U of S (Lab).
Note: Data points represent the mean out of 3 replicate measurements (n=15 per replicate). Error bars = 1 x SD.



A7-1. Study Process and Summary Decision Rules for Assessing Potential Risks of COIs in UCR Surface Water to White Sturgeon and Other Fish Species Having Similar Habitat Preferences and Food Habits.



- Upstream field site (UFS; City water intake of the City of Trail)
- Downstream Field Site I (DFSI; Waneta surface water sampling station; Exposure Site)
- Downstream Field Site II (DFSII; lower Marcus Flats; exposure site)

Figure B1-1. *In Situ* Study Sites Upstream and Downstream of Teck's Trail Facility.

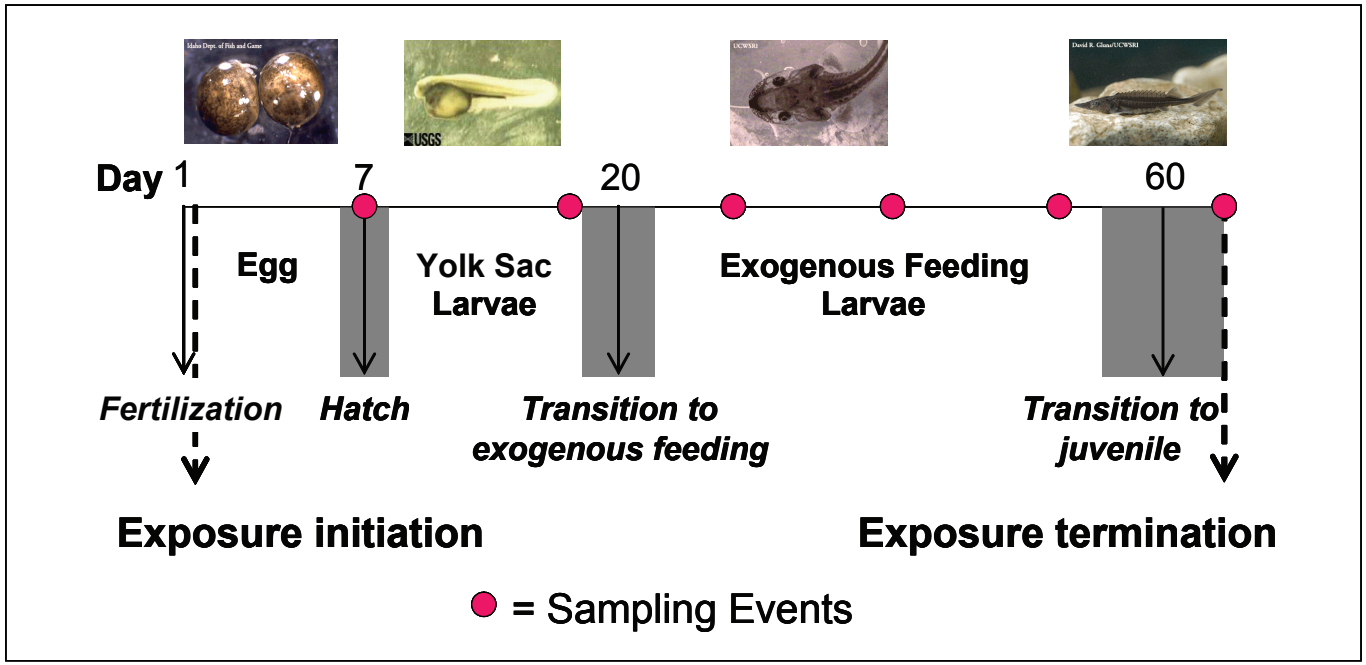


Figure B1-2. Sampling Times and Associated White Sturgeon Life Stages during the Exposure Experiments.

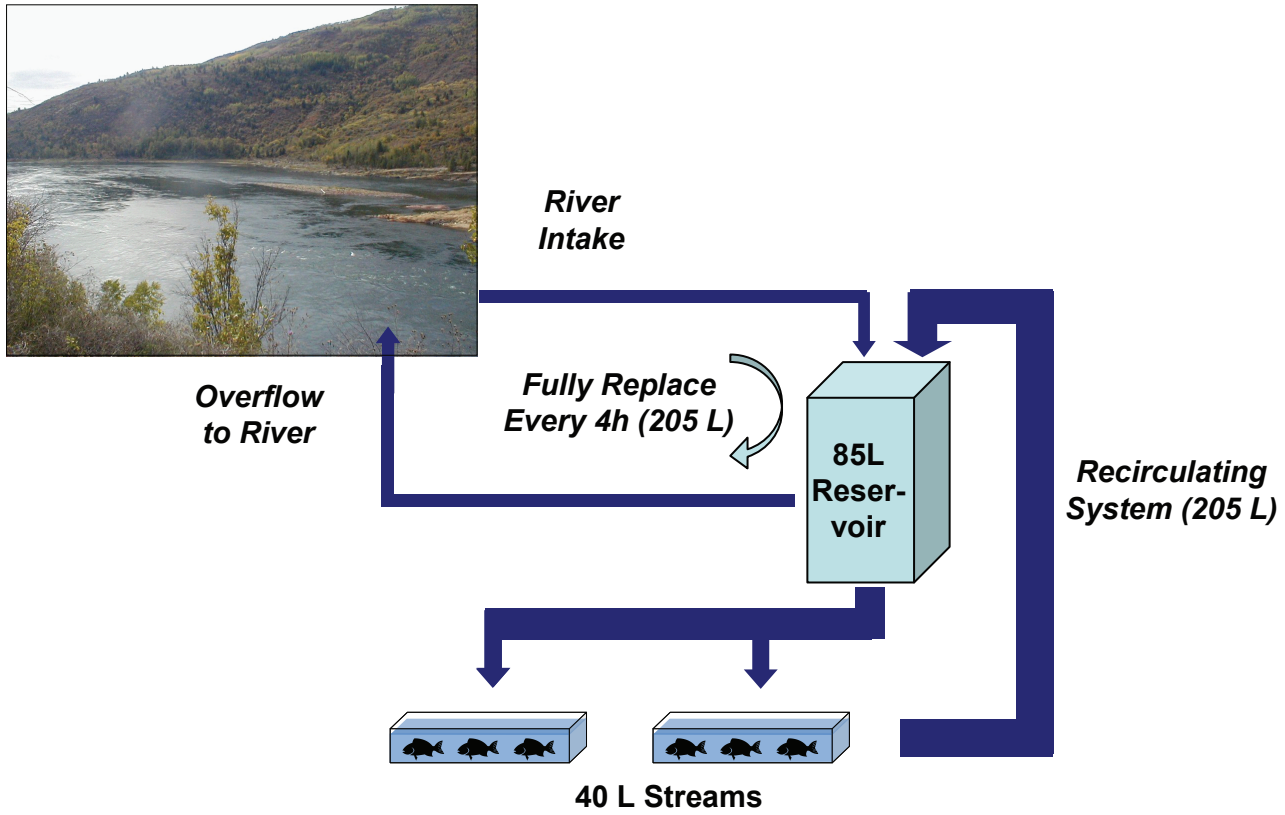


Figure B1-3. *In Situ* Exposure System and Experimental Layout of Study.
Note: Each exposure system consists of one 85 L reservoir and three 40 L exposure chambers. Each treatment group will consist of four replicate recirculating systems.

Table A5-1. Test Conditions and Endpoints for a 65-Day *In Situ* Toxicity Test with Early Life Stages of White Sturgeon (*A. transmontanus*) in 2009

Parameter	Conditions
Test Type	Whole-water toxicity test with river water upstream and downstream of Teck's Trail facility, and a clean laboratory water control
Temperature	15 ± 1 °C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	205 L recirculating flow through system each with three 40 L exposure chambers
Water Volume	205 L
Renewal Frequency of Water	Complete replacement of water (205 L) every 6 hours
Age of Organisms	4–8 hours post fertilization through ~60 days post hatch
Number of Organisms per Replicate Group	Exposure experiment will be initiated with 1,400–2,200 eggs per replicate treatment group; larvae will be thinned to 300 fish per test chamber (900 per replicate treatment group) after they hatch, and then further thinned to meet ASTM standards (ASTM E1241-05) throughout the experiment (target loading rates are not to exceed 0.5 g/L of solution passing through a system in 24 hours).
Number of Replicate Groups	Four replicate recirculating systems per replicate group
Feeding	Brine shrimp (*Artemia*) and ground blood worms 3 to 4 times per day; feeding will be initiated 2 to 3 days post swim-up to acclimate fry to food.
Aeration	None; recirculation of water provides sufficient aeration
Water Source	Columbia River water; dechlorinated and filtered city water
Test System Cleaning	Tanks gently siphoned and screens brushed if they become overgrown with organic matter.
Water Quality	Daily: dissolved oxygen, pH, temperature, and conductivity 1 to 3 times per week: ammonia, alkalinity, chlorine, hardness, nitrate, nitrite, sulfate, TOC, DOC. Frequency will be increased if marked changes in readings occur between measurements.
Test Duration	~65 days
Endpoints	Hatchability, survival, growth, gross morphology
Test Acceptability	Minimum hatching rate in the controls: 70 percent Minimum survival of fry until swim-up in the controls (~12-16 days post hatch): 80 percent Minimum survival during transition to feeding: 30 percent Minimum survival of fish post transition to feeding through end of experiment in the controls: 70 percent

Notes:

DOC = dissolved organic carbon
TOC = total organic carbon

Table A5-2. Mean Concentrations of Dissolved and Total Organic Carbon and Median Concentrations of Selected Dissolved COIs in Surface Water Up- and Downstream of Teck's Trail Facility and the Filtered City Water Control

	Number of Samples	Mean Concentration (mg/L) ± SD		Median Concentration (µg/L) (95th Percentile)		
		DOC	TOC	Cadmium	Copper	Lead
Upstream of Teck Trail Facility	14	1.2 ± 0.13	1.4 ± 0.07	0.04 (0.35)	1.0 (1.2)	0.15 (0.24)
Downstream of Teck Trail Facility	15	1.3 ± 0.12	1.4 ± 0.09	0.08 (1.9)	0.94 (1.2)	0.14 (0.54)
Filtered City Water Control	15	1.2 ± 0.08	1.2 ± 0.10	0.03 (0.45)	0.37 (0.98)	0.19 (1.2)
EPA CCC	--	--	--	0.21	7.1	2.1

Notes:

EPA CCC refers to the Chronic Continuous Criterion for fresh water species adjusted to the average hardness of 72 mg $CaCO_3$/L observed during the experiments (USEPA 2006b).

Number in parentheses is 95th percentile

COI = chemical of interest

DOC = dissolved organic compound

SD = standard deviation

TOC = total organic compound

Table A5-3. Test Conditions Used and Endpoints Measured during the 65-Day Toxicity Test with Early Life Stages of White Sturgeon (*A. transmontanus*) in 2008

Parameter	Conditions
Test Type	Laboratory dose-response toxicity study with laboratory water spiked with five concentrations each of cadmium, copper, and zinc
Temperature	15 ± 1 °C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	165 L recirculating flow through system each with two 40 L exposure chambers
Water Volume	165 L
Renewal Frequency of Water	Complete replacement of water (165 L) every 18 hours
Age of Organisms	1 day post fertilization through ~60 days post hatch
Number of Organisms per Replicate Group	Exposure experiment will be initiated with ~700 eggs per replicate treatment group; larvae will be thinned to 300 fish per test chamber (600 per replicate treatment group) after they hatch, and then further thinned to meet ASTM standards (ASTM E1241-05) throughout the experiment (target loading rates are not to exceed 0.5 g/L of solution passing through a system in 24 hours).
Number of replicate Groups	Metal exposure group Two replicate recirculating systems Control group: Four replicate recirculating systems
Feeding	Brine shrimp (*Artemia*) and ground blood worms 3 to 4 times per day; feeding will be initiated 2 to 3 days post swim-up to acclimate fry to food.
Aeration	None; recirculation of water provides sufficient aeration
Water Source	Dechlorinated and filtered city water
Test System Cleaning	Tanks gently siphoned and screens brushed if they become overgrown with organic matter
Water Quality	Daily: dissolved oxygen, pH, temperature, and conductivity 1 to 3 times per week: ammonia, alkalinity, chlorine, hardness, nitrate, nitrite, sulfate. Frequency will be increased if marked changes in readings occur between measurements.
Test Duration	~65 days
Endpoints	Hatchability, survival, growth, gross morphology
Test Acceptability	Minimum hatching rate in the controls: 70 percent Minimum survival of fry until swim-up in the controls (~12–16 days post hatch): 80 percent Minimum survival during transition to feeding: 30 percent Minimum survival of fish post transition to feeding through end of experiment in the controls: 70 percent

Table A5-4. Median Lethal Concentrations and Lowest- and No-Observed-Adverse-Effect Concentrations Determined for Early Life Stages of Sturgeon after Exposure to Cadmium, Copper, and Zinc for ~60 Days post Hatch

	LC50 (µg/L)	LOAEC (µg/L)	NOAEC (µg/L)	EPA CMC (µg/L)
Cadmium	6.8	10	1.3	0.21
Copper	16	22	3.6	7.1
Zinc	360	220	36	91

Notes:

EPA CMC refers to the Criteria Maximum Concentration for fresh water species adjusted to the average hardness of 72 mg $CaCO_3$/L observed during the experiments (USEPA 2006b).

Concentrations represent nominal concentrations and had not been confirmed by ICP/MS at the time this QAPP was written.

LC50 = median lethal concentration
LOAEC = lowest-observed-adverse-effect concentration
NOAEC = no-observed-adverse-effect concentration

Table A5-5. Test Conditions Used and Endpoints Measured during the 96-Hour Acute Toxicity Test with White Sturgeon (*A. transmontanus*) in 2008

Parameter	Conditions
Test Type	Static renewal whole-water toxicity test with river water upstream of Teck's Trail facility, and filtered laboratory water
Temperature	16 ± 1 °C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	Static renewal system
Water Volume	0.5 L
Renewal Frequency of Water	50 percent test solution renewal every 12 hours; pre-equilibration of test solutions ≥ 48 hours prior to water change
Age of Organisms	8-10 days post hatch
Number of Organisms per Replicate Group	15
Number of Replicate Groups	Four replicates per treatment group
Feeding	n/a (yolk sac larvae)
Aeration	None
Water Source	Columbia river water; dechlorinated and filtered city water
Water Quality	Dissolved oxygen, pH, temperature, and conductivity; ammonia, alkalinity, chlorine, hardness and major cations (Ca, Mg, Na, K), nitrate, nitrite, sulfate, TOC, DOC, COIs
Test Duration	96 hours
Endpoints	Survival
Frequency of observations	Observations will be made after 0, 6, 12, and 24 hours, and every 24 hours thereafter.
Test Acceptability	See Table B1-7.

Notes:
COI = chemical of interest
DOC = dissolved organic carbon
TOC = total organic carbon

Table A5-6. Median Lethal Concentrations and Least- and No-Observed-Adverse-Effect Concentrations Determined for Early Life Stages (~8-10 Days Post Hatch) of Sturgeon after Exposure to Cadmium, Copper, and Zinc for 96 Hours in a Static Renewal System

	LC50 (µg/L)		LOAEC (µg/L)		NOAEC (µg/L)		
	Lab	*In Situ*	Lab	*In Situ*	Lab	*In Situ*	WER
Cadmium	16	62	31	130	7.8	31	0.25
Copper	48	35	25	25	6.3	6.3	1.4
Zinc	156	645	310	1,300	130	310	0.24

Notes:

Concentrations represent nominal concentrations.

LC50 = median lethal concentration
LOAEC = lowest-observed-adverse-effect concentration
NOAEC = no-observed-adverse-effect concentration
WER = water effects ratio

Table A7-1. Possible Decision Outcomes in Testing the Statistical Hypotheses (H_0) Associated with the Studies

True State of Nature	Don't Reject H_0	Reject H_0
H_0	Correct Decision Pr(No Error) = $(1 - \alpha)$	Incorrect Decision Pr(Type I Error) = α
H_1	Incorrect Decision Pr(Type II Error) = β	Correct Decision Pr(No Error) = $(1 - \beta)$

Table A7-2. Number of Replicate Groups Required (n) To Detect Significant Differences among Sites, and Percent Difference Detectable with an n of Four

	α=0.05; β=0.2		α=0.05; β=0.1		α=0.1; β=0.2		α=0.1; β=0.1	
Difference	n	Power	n	Power	n	Power	n	Power
5%	7	85%	8	90%	6	87%	7	92%
10%	3	90%	3	90%	3	97%	3	97%
20%	2	96%	2	96%	2	100%	3	100%
Detectable difference at n = 4	7%		8%		6%		7%	

Notes:

Calculations were conducted under consideration of the means and variances for mortalities observed in the different treatment groups during the 2008 surface water toxicity studies with early life stages of white sturgeon (Section A5.2.1)

Table B1-1. Sampling Design for the 2009 Water Toxicity Studies with Early Life Stages of White Sturgeon and ASTM (2005) Target Values

Parameter	2009 Sturgeon Water Toxicity Study	ASTM (2005) Target Values
Time of exposure initiation	≤ 12 hours	Salmonids: ≤ 96 hours; All other species: ≤ 48 hours
Exposure duration	66 days (>40 days post swim-up)	Salmonids: ≥ 30 days post swim-up; Pike: 32 days; Fathead minnow: ≥ 28 days; White sucker: 32 days; Channel catfish: 32 days; Bluegill: 32 days
Loading density/rate	≤ 0.2 g/L per 24 hours	≤ 0.5 g/L per 24 hours
Number of true replicates per treatment/dose	4	≥ 2
Number of fish per treatment (controls) at end of study	≥ 80	≥ 40
Observations	≥ 2 times per day	≥ 1 time per day
Feeding	≥ 3 times per day	≥ 1 time per day

Table B1-2. Test Conditions for a 66-Day Toxicity Test with Early Life Stages of White Sturgeon (*A. transmontanus*)

Parameter	Conditions
Test Type	Whole-water toxicity test with river water upstream and downstream of Teck's Trail facility, and a clean laboratory water control
Temperature	15 ± 1°C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	205 L recirculating flow through system each with three 40 L exposure chambers.
Water Volume	205 L
Renewal Frequency of Water	Complete replacement of water (205 L) every 6 hours.
Age of Organisms	8-12 hours post fertilization through ~60 days post hatch
Number of Organisms per Replicate Group	Exposure experiment will be initiated with 2,000 eggs per replicate treatment group; Larvae will be thinned to 200 fish per test chamber (600 per replicate treatment group) after they hatch, and then further thinned to 150 fish per test chamber prior to initiation of self-feeding in accordance with the optimized study design based on the 2008 surface water toxicity studies (Entrix, in prep).
Number of Replicate Groups	Four replicate recirculating systems per replicate group
Feeding	Brine shrimp (*Artemia*) and ground blood worms 3 to 4times per day *ad libidum*; feeding will be initiated 2 to 3 days post swim-up to acclimate fry to food. Note: Food will be analyzed for presence of COIs prior to initiation of studies.
Aeration	None; recirculation of water provides sufficient aeration
Water Source	Columbia River water; dechlorinated and filtered city water
Test System Cleaning	Tanks gently siphoned and screens brushed if they become overgrown with organic matter.
Water Quality	Daily: dissolved oxygen, pH, temperature, and conductivity 1 to 3 times per week: Ammonia, alkalinity, chlorine, hardness and major cations (Ca, Mg, Na, K), nitrate, nitrite, sulfate, TOC, DOC, COIs. Frequency will be increased if marked changes in readings occur between measurements.
Test Duration	~66 days
Endpoints	Hatchability, survival, growth, gross morphology
Test Acceptability	Minimum hatching rate in the controls/reference should be 60 percent Minimum survival of fry until swim-up in the controls/reference (~12-16 days post hatch) should be 80 percent Minimum survival of fish post swim-up through end of experiment in the controls/reference: 30 percent

Notes:

COI = chemical of interest
DOC = dissolved organic carbon
TOC = total organic carbon

Table B1-3. General Activity Schedule for Conducting a 66-Day Toxicity Test with Early Life Stages of White Sturgeon (*A. transmontanus*)

Day	Conditions
–52 to –15	• Set up of trailers and recirculating exposure systems upstream and downstream of Teck's Trail facility and in the area of Marcus Flats in the U.S. • Establish power and city water delivery at all sites • Establish field laboratories for sampling and water quality measurements • Adjust exposure system life-feed and flush exposure systems with test water
–14 to –1	• Begin water quality monitoring • Take weekly samples for metal analysis • Analyze dissolved metal concentrations in water samples
Hatchability Test	
0	• Test water quality in each exposure system (dissolved oxygen, pH, temperature, ammonia, nitrate, nitrite, hardness, alkalinity, phosphate, chlorine, sulfate) • Acclimate freshly fertilized eggs to water temperature in exposure systems • Transfer eggs to egg hatching jars • Adjust flow-through egg hatching jars so that the top layer of eggs roles very gently but do not get pushed up in the water column
1 to 6	• Observe systems with hatching jars 2 to 3 times per day • Measure dissolved oxygen, temperature, pH, and conductivity daily
3	• Ensure that neurolation has been completed • Increase water flow to hatching jars such that eggs are vigorously circulated throughout the jar • Test water quality in each exposure system
7 to 9	• Observe and record hatching activities • Count and transfer hatched larvae to main exposure chambers • Count and remove dead eggs from hatching jars
Pre-Swim-Up Test	
7 to 20	• Observe larvae 2 or 3 times per day • Measure dissolved oxygen, temperature, pH, and conductivity daily and all other water quality parameters at least every 5 days • Count, weigh and remove dead fry and preserve dead fish in formalin • Observe and record any behavioral abnormalities • Observe and record swim-up and presence/rejection of black yolk sac plugs
11	• Thin fish to 200 individuals per exposure chamber (690 fish per treatment group/system)
18	• Thin fish to 150 individuals per exposure chamber, and begin adding *Artemia* and bloodworms to the chambers to condition fry to food
Post-Swim-Up Test	
21 – 25	• Record swim-up and feeding behavior of fry • Thin fish to 130 individual per treatment chamber (600 per treatment system/group) • Record weight and length of fry removed for thinning purposes and fix these fish in formalin
21 – 65	• Feed fish 4-times per day • Clean systems daily • Record and remove mortalities daily, and measure, weigh and fix dead fish in formalin • Test water quality in each exposure system (Daily: dissolved oxygen, pH, temperature, conductivity; Weekly: ammonia, nitrate, nitrite, hardness, alkalinity, phosphate, chlorine, sulfate, TOC, DOC, metals)
31 to 35	• Thin fish to 100 individual per treatment chamber • Record weight and length of fry removed for thinning purposes and fix these fish in formalin

Table B1-3. General Activity Schedule for Conducting a 66-Day Toxicity Test with Early Life Stages of White Sturgeon (*A. transmontanus*)

Day	Conditions
41 to 45	• Record weight and length of fry removed for thinning purposes and fix these fish in formalin
51 to 55	• Thin fish to 80 individual per treatment chamber • Record weight and length of fry removed for thinning purposes and fix these fish in formalin
66	• Terminate study • Measure complete water quality suite including metals • Euthanize fish in MS222 • Measure and weigh fish • Fix fish in formalin for later analysis

Notes:

DOC = dissolved organic carbon
TOC = total organic carbon

Table B1-4. Test Acceptability Requirements for a 66-Day Toxicity Test with Early Life Stages of White Sturgeon (*A. transmontanus*)

	Acceptance Criteria
1	Freshly fertilized eggs from at least 2–4 different females and males are to be use (time between hatching and initiation of study must not exceed 24 hours
2	Average hatching rate of eggs in the lab water controls should not to be less than 70 percent
3	Average survival of fry until swim-up in the lab water controls should be greater or equal to 80 percent
4	Average survival of fry post swim-up in the lab water controls should be greater or equal to 30 percent
5	All water quality parameters with the exception of dissolved oxygen and temperature should not vary by more than 50 percent during the exposure
6	Dissolved oxygen should be maintained above 80 percent saturation
7	Average daily temperature should be maintained at 15 ± 1°C; the instantaneous temperature must always be within ± 3°C of 15°C
Additional Acceptance Criteria	
1	All organisms must be from the same source
2	Survival and hatchability in the lab water controls should be comparable to those observed at the Kootenay Trout Hatchery for the fish from the same fertilization event
3	All test systems and chambers should be identical and should be run under the same re-circulating conditions for each study.
4	Natural physico-chemical conditions of the control lab water should be within the tolerance limit for white sturgeon early life stages
5	Food used for both studies should be obtained from the same source and should have been tested for possible comparability with white sturgeon early life stages prior to initiation of the studies

Table B1-5. Test Conditions for Conducting a 96-Hour Acute Toxicity Test with White Sturgeon (*A. transmontanus*) Using Site and Lab Water

Parameter	Conditions
Test Type	Static renewal whole-water toxicity test with river water upstream of Teck's Trail facility, and filtered laboratory water
Temperature	16 ± 1 °C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	Static renewal system
Water Volume	0.5 L
Renewal Frequency of Water	50 percent test solution renewal every 12 hours; pre-equilibration of test solutions ≥ 48 hours prior to water change.
Age of Organisms	8-10 days post hatch
Number of Organisms per Replicate Group	15
Number of Replicate Groups	Four replicates per treatment group
Feeding	n/a (yolk sac larvae)
Aeration	None
Water Source	Columbia River water; dechlorinated and filtered city water
Water Quality	dissolved oxygen, pH, temperature, and conductivity; ammonia, alkalinity, chlorine, hardness and major cations (Ca, Mg, Na, K), nitrate, nitrite, sulfate, TOC, DOC, COIs
Test Duration	96 hours
Endpoints	Survival
Frequency of observations	Observations will be made after 0, 6, 12, and 24 hours, and every 24 hours thereafter
Test Acceptability	See Table B1-7

Notes:
COI = chemical of interest
DOC = dissolved organic carbon
TOC = total organic carbon

Table B1-6. General Activity Schedule for Conducting a 96-Hour Acute Toxicity Test with White Sturgeon (*A. transmontanus*)

Hour	Conditions
–48	• Prepare test solutions, and allow to completely equilibrate for 48 hours • Set up exposure systems
–24 to –1	• Acclimate of sturgeon to exposure system containing only reference/control water.
0	• Initiate exposure by adding appropriate concentrations of test solutions to all treatment chambers • Test water quality in each exposure system (dissolved oxygen, pH, temperature, ammonia, nitrate, nitrite, hardness, alkalinity, phosphate, chlorine, sulfate) • Take and preserve samples for COI analysis
6	• Observe all exposure groups and record changes in behavior (e.g., lethargy, hyperactivity) and mortalities
12	• Observe all exposure groups and record changes in behavior (e.g. lethargy, hyperactivity) and mortalities • Replace 50 percent of test solutions
24	• Test water quality in each exposure system (dissolved oxygen, pH, temperature) • Observe all exposure groups and record changes in behavior (e.g., lethargy, hyperactivity) and mortalities • Replace 50 percent of test solutions
36	• Replace 50 percent of test solutions
48	• Test water quality in each exposure system (dissolved oxygen, pH, temperature) • Observe all exposure groups and record changes in behavior (e.g., lethargy, hyperactivity) and mortalities • Replace 50 percent of test solutions
60	• Replace 50 percent of test solutions
72	• Test water quality in each exposure system (dissolved oxygen, pH, temperature) • Observe all exposure groups and record changes in behavior (e.g., lethargy, hyperactivity) and mortalities • Replace 50 percent of test solutions
84	• Replace 50 percent of test solutions
96	• Observe all exposure groups and record changes in behavior (e.g., lethargy, hyperactivity) and mortalities • Terminate study • Measure complete water quality suite and preserve samples for COI analyses • Euthanize surviving fish in MS222

Notes:

COI = chemical of interest

Table B1-7. Test Acceptability Requirements for a 96-Hour Acute Toxicity Test with White Sturgeon (*A. transmontanus*)

	Acceptance Criteria
1	Fish used for test should be from at least 2–4 different parent females and males
2	Average hatching rate of eggs in the lab water controls should not to be less than 70 percent
3	Average survival of fry until test initiation should be greater or equal to 70 percent
4	Average survival in controls should be greater or equal to 80 percent
5	All water quality parameters with the exception of dissolved oxygen and temperature should not vary by more than 50 percent during the exposure
6	Dissolved oxygen should be maintained above 80 percent saturation
7	Average daily temperature should be maintained at 16 ± 1°C; the instantaneous temperature must always be within ± 3 °C of 16°C
Additional Acceptance Criteria	
1	All organisms must be from the same source

Table B1-8. Test Conditions for Conducting a 96-Hour Acute Toxicity Test with White Sturgeon (*A. transmontanus*) Using Lab Water Only

Parameter	Conditions
Test Type	Static renewal whole-water toxicity test with filtered laboratory water
Temperature	16 ± 1 °C
Light Quality	Wide spectrum fluorescent lights
Photoperiod	16 hours light; 8 hours dark
Test System	Static renewal system
Water Volume	0.5 L
Renewal Frequency of Water	50 percent test solution renewal every 12 hours; pre-equilibration of test solutions ≥ 48 hours prior to water change
Age of Organisms	8–10 days post hatch
Number of Organisms per Replicate Group	15
Number of Replicate Groups	Four replicates per treatment group
Feeding	Yolk sac larvae: n/a; 2 times per day just prior to water change (remove food residues before water change)
Aeration	None
Water Source	Dechlorinated and filtered laboratory water
Water Quality	Dissolved oxygen, pH, temperature and conductivity; ammonia, alkalinity, chlorine, hardness and major cations (Ca, Mg, Na, K), nitrate, nitrite, sulfate, TOC, DOC, COIs
Test Duration	96 hours
Endpoints	Survival
Frequency of Observations	Observations will be made after 0, 6, 12, and 24 hours, and every 24 hours thereafter.
Test Acceptability	See Table B1-7

Notes:

COI = chemical of interest
DOC = dissolved organic carbon
TOC = total organic carbon

Table B2-1. Recommended Laboratory Methods for Analysis of Surface Water Samples

	Sample Preparation		Quantitative Analysis	
Analytes	Protocol	Procedure	Protocol	Procedure
Conventional Parameters				
Alkalinity as $CaCO_3$	--	--	SM 2320B	Titrimetric
DOC	SM 5310C	Filtration, chemical oxidation	SM 5310C	Infrared detector
Hardness as $CaCO_3$	--	--	SM 2340C	Titrimetric
TDS/TSS	--	--	SM 2540	Gravimetric
TOC	SM 5310C	Filtration, chemical oxidation	SM 5310C	Infrared detector
pH	EPA 150.1/SM 4500 H^+ B	--	EPA 150.1/SM 4500 H^+ B	Electrometric
Silicon dioxide (silica) (dissolved)	EPA 370.1	Filtration	EPA 370.1	Colorimetric
Major Ions				
Calcium, magnesium, potassium, sodium	EPA 3005	Acid digestion	EPA 6010B	ICP/AES
Chloride, fluoride, sulfate	--	--	EPA 300.0	Ion chromatography
Nutrients				
Ammonia	SM 4500-NH3 G	Buffered to pH 9.5	SM 4500-NH3 G	Colorimetric
Nitrate, nitrite	--	--	EPA 300.0	Ion chromatography
Total phosphorus	EPA 365.3	Persulfate digestion	EPA 365.3	Colorimetric
Common Metals and Metalloids [a]				
Aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, nickel, selenium, silver, thallium, vanadium, zinc	EPA 3005	Acid digestion	EPA 6020	ICP/MS
Iron	EPA 3005	Acid digestion	EPA 6010B	ICP/AES
Mercury	EPA 1631E	BrCl oxidation	EPA 1631E	AFS
Other Metals and Metalloids [a]				
Strontium, titanium	EPA 3005	Acid digestion	EPA 6010B	ICP/AES

Notes:

AFS = atomic fluorescence spectroscopy
CVAAS = cold vapor atomic absorption spectrometry
DOC = dissolved organic carbon
EPA = U.S. Environmental Protection Agency
ICP/AES = inductively coupled plasma/atomic emission spectrometry
ICP/MS = inductively coupled plasma/mass spectrometry
SM = Standard Methods for the Examination of Water and Wastewater
TBD = to be determined
TDS = total dissolved solids
TOC = total organic carbon
TSS = total suspended solids

[a] Water samples will be collected and analyzed for total and dissolved metals and metalloids.

Table B2-2. Required Sample Containers, Preservation, and Holding Times

	Container [a]		Preservation	Holding Time	Proposed Laboratory Sample Size [b]
	Type	Size			
Conventional Parameters					
Alkalinity as $CaCO_3$	HDPE	250 mL	4±2ºC	28 days	50 mL
Dissolved organic carbon	HDPE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	20 mL
Hardness as $CaCO_3$	HDPE	250 mL	4±2ºC	28 days	50 mL
Total dissolved solids	HDPE	500 mL	4±2ºC	7 days	200 mL
Total suspended solids	HDPE	500 mL	4±2ºC	7 days	200 mL
Total organic carbon	HDPE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	20 mL
Silicon dioxide (silica) (dissolved)	HDPE	100 mL	4±2ºC	28 days	50 mL
Major Ions					
Calcium, magnesium, potassium, sodium	HDPE	250 mL	HNO_3 to pH <2; 4±2ºC	28 days	60 mL
Chloride, fluoride, sulfate	HDPE	250 mL	4±2ºC	48 hours	60 mL
Nutrients					
Ammonia	HPDE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	5 mL
Nitrate, nitrite	HDPE	250 mL	4±2ºC	28 days	60 mL
Total phosphorus	HDPE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	100 mL
Common metals and metalloids [c]	HDPE	Two 1 L	5 mL of 1:1 HNO_3; 4±2ºC	6 months	1 L
Mercury	FP or G w/ FP-lined lids	500 mL	BrCl in lab within 28 days of collection; 4±2ºC	90 days	500 mL
Other metals and metalloids [c]	HDPE	Two 250 mL	HNO_3 in lab within 28 days of collection; 4±2ºC	6 months	250 mL

Notes:

FP = fluoropolymer
G = glass
HDPE = high density polyethylene bottle

[a] Sample container sizes may be modified to meet laboratory requirements
[b] Extra sample volume will be collected at a frequency of 5 percent of samples to accommodate requirements for laboratory quality control samples
[c] Surface water samples will be collected and analyzed for total and dissolved metals and metalloids. A total of 2 L of water will be collected for the common metals/metalloids analyses (1 L each for total and dissolved), and 500 mL will be collected for analysis of mercury.

Table B5-1. Experimental Quality Control Samples for Precision and Accuracy

Type of QC Sample	Frequency	Acceptance Criteria
Equipment rinsate blank	1 per week per equipment type	No analyte should be detected at >3 times the laboratory blank
Matrix spike/matrix spike duplicate (MS/MSD)	1 per 20 samples	RPD should be ≤30 percent for each analyte.
Experimental blank	1 per sampling event	No analyte should be detected at >3 times the laboratory blank.

Note:
MS/MSD samples are included as experimental quality control samples for planning purposes, to ensure sufficient sample volume is collected for the analyses.

Table D2-1. Data Validation Qualifiers

Qualifier	Explanation of Qualifier
U	The compound was analyzed for, but was not detected above the reported method detection limit.
J	The analyte was positively identified; the associated numerical value is the approximate concentration of the analyte in the sample.
R	The sample results are rejected due to serious deficiencies in the ability to analyze the sample and meet quality control criteria. The presence or absence of the analyte cannot be verified.
UJ	The analyte was not detected above the reported method detection limit. However, the reported limit is approximate and may or may not represent the actual limit of quantitation necessary to accurately and precisely measure the analyte in the sample.
B	The analyte was positively identified; the reported concentration is greater than the instrument detection limit, but less than the QAPP-specified reporting limit.

APPENDIX A

SUMMARY OF KOOTENAI RIVER WHITE STURGEON STUDIES

INFORMATION SHEET

Summary of Kootenai River White Sturgeon Studies
U. S. Fish and Wildlife Service
Upper Columbia Fish and Wildlife Office
Spokane, Washington
2007/2008
Contacts: Julie Campbell or Toni Davidson (509) 891-6839

Why conduct studies on Kootenai River white sturgeon?

Many contributing factors are of concern for the lack of recruitment (addition of individuals via natural reproduction) into the wild Kootenai River white sturgeon population. The evaluation of effects of potential chemical exposures to white sturgeon in the Kootenai River is necessary to support ongoing recovery efforts, and is identified as a research need in the Recovery Plan for the Kootenai River Population of White Sturgeon. Other sturgeon species (Atlantic sturgeon, shortnose sturgeon) have been shown to be very sensitive to chemical exposures relative to other fish species (Dwyer et al. 2005). The studies described herein focus specifically on white sturgeon, and evaluate potential effects to the fish from chlorine and copper in the Kootenai and Columbia Rivers, as well as three herbicides proposed for control of Eurasian watermilfoil in the Kootenai River. At this time we do not know how laboratory results relate to the potential effects to wild Kootenai River and Columbia River white sturgeon populations because of potential dilution, bioavailability, actual river concentrations, or other factors specific to each river system.

The objectives of these studies are to:

- Evaluate toxic effect levels of chlorine, copper and 3 herbicides on sensitive life stages of sturgeon in the laboratory;
- Evaluate actual contributions of municipal discharges into the Kootenai River (i.e., measure concentrations of chlorine and metals in river) and compare to sturgeon toxicity data;
- Compare recommended application rates of herbicides to sturgeon toxicity data.

2007 Laboratory Studies: acute toxicity to early life stage and juvenile white sturgeon from Chlorine and Copper (Tables 1 and 2)

The objectives of the studies are to:
- Identify toxic concentrations of chlorine and copper to sturgeon at 30 days post swim-up (dps) and 5-6 months - complete;
- Identify toxic concentrations of chlorine and copper to rainbow trout (as above) and compare to sturgeon toxicity – complete;
- Compare toxic effect concentrations to measured field concentrations in Kootenai River – planned for spring/summer 2008.

Study Initiated in July 2007
Funding Source: USFWS, USGS partnership

Results:

Table 1. Chlorine LC50[a] (96 hr) estimates for Kootenai River (KRWS) and Columbia River white sturgeon (CRWS) and rainbow trout (RBT) at 30 and 160 days post swim-up (dps) (standard deviations for fish weight and length in parentheses and 95% confidence intervals for LC50s in parentheses) (Little 2008a).

Species	Lifestage (dps)	Number of replicates per treatment	Average Fish Weight (gm)	Average Fish Length (cm)	LC50 [a] µg/L, observed
KRWS	30	4	0.07 (0.01)	2.4 (0.14)	42.3 (36.6 – 48.1)
CRWS	160	2	18.1 (5.2)	17.0 (2.3)	34.3 (28.3 – 40.4)
RBT	30	4	0.07 (0.01)	2.4 (0.1)	104 (89.1 – 118)
RBT	160	2	4.8 (2.4)	7.4 (1.2)	287 (152 – 423)

Note: There were five fish per replicate treatment.
[a]LC50 – concentration that results in 50% mortality of test population.

Table 2. Copper LC50 [a] (96 hour) estimates for white sturgeon and rainbow trout at 30 and 160 days post swim-up (dps) (standard deviations for fish weight and length in parentheses and 95% confidence intervals for LC50s in parentheses) (Little 2008b).

Species	Life stage (dps)	Average Fish Weight (gm)	Average Fish Length (cm)	LC50 [a] µg/L, based on measured concentrations	1996 Hardness-dependent acute WQC[b] for Copper (µg/L)
KRWS	30	0.07 (0.01)	2.4 (1.4)	3.1 (2.6 – 3.7)	12
CRWS	30	0.08 (12.7)	2.5 (1.6)	4.9[c] (3.3 – 6.4)	12
CRWS	160	18.1 (5.2)	17.0 (2.3)	245 (189 –300)	10
RBT	30	0.17 (0.02)	2.9 (0.84)	71.1 (58.6 – 83.7)	15
RBT	160	4.5 (2.3)	7.2 (12.1)	125[d] (72.7– 178)	15

[a]LC50 – concentration that results in 50% mortality of test population.
[b]Acute Water Quality Criteria (WQC) for copper at average water hardness for all tests (90.5 mg/L) is 13 µg/L.
[c]LC50 value based copper concentrations measured at the end of the KRWS exposure that immediately preceded the CRWS exposure.
[d]Copper concentrations at 96 hours were approximately 50% of that observed at time zero. We are reasonably certain this was caused by an injection pump malfunction that occurred following a power outage after 72 hours of the test. Because mortality was clustered within the first 72 hours of exposure, we consider the time zero concentrations to be most appropriate for the calculation of the 96-h LC50.

2008 Chlorine Field Investigation

The objectives of the study are to:

- Evaluate concentrations of chlorine and copper/metals in Kootenai River (surface water);
- Sample water column at surface and at depth along 3 transects across the Kootenai River (upstream of municipal treatment plant outfalls, between outfalls, downstream of outfalls);
- Two sampling events (May, August);
- Compare concentrations to acute toxicity data for sturgeon from laboratory toxicity studies (above).

Field collection scheduled for Spring/Summer 2008; study will be completed in 2009.
Funding Source: USFWS

2007 Laboratory Studies: acute toxicity to early life stage and juvenile white sturgeon from three herbicides (Tables 3, 4 and 5)

The objectives of the study are to:

- Establish toxic concentrations of triclopyr (trade name Renovate), fluridone (trade name Sonar) and 2,4-D (as DMA-4) to sturgeon at 30 dps and 5-6 months - complete;
- Evaluate acute toxicity of triclopyr, fluridone and 2,4-D to rainbow trout (as above) and compare to sturgeon toxicity - complete;
- Compare toxicity data to proposed application rates for Kootenai River system – complete.

Study Initiated in August 2007
Funding Source: USFWS, USGS, SePRO Corp., 2,4-D Task Force partnership

Results:

Table 3. Observed concentration (mg/L) of **triclopyr** (as acid equivalent) and percent mortality of Kootenai River white sturgeon (KRWS) and rainbow trout (RBT) observed during exposure at 30 and 160 days post swim-up (dps) to Renovate 3 (Little 2008c).

Treatment	Species	Exposure Concentration, mg/L (standard error in parentheses)		Percent Mortality (96 h) (standard error in parentheses) [loss of equilibrium]	
		30 dps	160 dps	30 dps	160 dps
KRWS	Control	<0.03	<0.03	0	0
	Low	0.5 (0.1)	55.0 (1.0)	0	0
	Med-Low	2.0 (0.1)	NC[b]	0	NC[b]
	Med	2.1 (0.1)	107 (0.6)	0	15 (0.96)
	Med-Hi	4.35 (0.5)	NC[b]	0	NC[b]
	Hi	8.0 (0.3)	191 (1.1)	0 [100% LOE[a]]	100
RBT	Control	<0.03	<0.03	0	0
	Low	13.5 (0)	74.7 (1.2)	0	0
	Med-Low	NC[b]	NC[b]	0	NC[b]
	Med	49.2 (0.3)	154 (1.8)	0	0
	Med-Hi	NC[b]	NC[b]	0 [100% LOE[a]]	NC[b]
	Hi	159 (4.6)	264 (23.1)	100	0

[a]LOE -Loss of equilibrium (fish could not maintain balance and turned upside down)
[b]NC – Not Conducted

Triclopyr data summary:

- No observed KRWS or RBT effects or mortality associated with triclopyr exposure near or within maximum recommended application rate of 2.5 mg/L;
- 30 dps KRWS: Lowest observed effect concentration (LOEC) was 8.0 mg/L based on loss of equilibrium;
- 160 dps KRWS: LC50 calculated at 111 mg/L;
- 30 dps RBT: LOEC observed at nominal concentration of 75 mg/L based on loss of equilibrium;
- 160 dps RBT: LC50 calculated at >264 mg/L.

Table 4. Observed concentration (mg/L) of **fluridone** (as active ingredient) and percent mortality of Kootenai River white sturgeon (KRWS) and rainbow trout (RBT) observed during exposure at 30 and 160 days post swim-up (dps) to Sonar A.S. (Little 2008c).

Treatment	Species	Exposure Concentration, mg/L (standard error in parentheses)		Percent Mortality (96 h) (standard error in parentheses) [loss of equilibrium]	
		30 dps	160 dps	30 dps	160 dps
KRWS	Control	<0.016	<0.016	0	0
	Low	0.07 (0.08)	0.52 (0.42)	5 (0.5)	0[100% LOE[a]]
	Med-Low	0.12 (0.04)	NC[b]	0	NC[b]
	Med	0.22 (0.08)	0.72 (0.59)	5 (0.5)	0[100% LOE[a]]
	Med-Hi	0.47 (0.04)	NC[b]	30 (0.58)	NC[b]
	Hi	0.88 (0.06)	1.34 (1.18)	20 (0.82)	0[100% LOE[a])
RBT	Control	<0.016	<0.016	0	0
	Low	0.67 (0.27)	1.83 (1.4)	0	0[100% LOE[a]]
	Med-Low	NC[b]	NC[b]	0	NC[b]
	Med	2.7 (1.02)	3.74 (2.8)	0	0[100% LOE[a]]
	Med-Hi	NC[b]	NC[b]	0	NC[b]
	Hi	8.81 (5.1)	7.01 (5.7)	45(2.22) [55% LOE[a]]	5 (0.5) [100% LOE[a]]

[a]LOE -Loss of equilibrium
[b]NC – Not Conducted

Fluridone data summary:
- Mortality (5%) of KRWS exposed to fluridone was observed below the maximum recommended application rate of 0.150 mg/L;
- No observed RBT mortality was observed associated with fluridone exposure near or within maximum recommended application rate;
- 30 dps KRWS: LC50 calculated at 1.61 mg/L;
- 160 dps KRWS: LOEC observed at 0.52 mg/L based on loss of equilibrium;
- 30 dps RBT: LOEC observed at 8.8 mg/L based on loss of equilibrium;
- 160 dps RBT: LOEC observed at 1.8 mg/L based on loss of equilibrium.

Table 5. Observed concentration (mg/L) of **2,4-D** (as acid equivalent) and percent mortality of Kootenai River white sturgeon (KRWS) and rainbow trout (RBT) observed during exposure at 30 and 160 days post swim-up (dps) to DMA 4 IVM (Little 2008d).

Species	Treatment	Exposure Concentration, mg/L (standard error in parentheses)		Percent Mortality (96 h) (standard error in parentheses) [loss of equilibrium]	
		30 dps	160 dps	30 dps	160 dps
KRWS	Control	< 0.074	2.4 (4.7)	0	0
	Low	1.22 (0.05)	19.8(0.1)	5 (0.5)	0
	Med-Low	2.5 (0)	NC[b]	5 (0.5)	NC[b]
	Med	4.98 (0.4)	38.9 (0.5)	15 (0.5)	0[37.5% LOE[a]]
	Med-Hi	10 (0)	NC[b]	15 (0.96)	NC[b]
	Hi	42.3 (4.5)	85.3 (0.3)	65 (1.7)	0[100% LOE[a]]
RBT	Control	< 0.074	< 0.074	0	0
	Low	54.1 (0.07)	77.2 (0.2)	0	0
	Med-Low	187.5 (1.3)	NC[b]	0	NC[b]
	Med	200 (3.5)	176.7 (0.6)	0	0
	Med-Hi	387 (8.4)	NC[b]	5 (0.5)	NC[b]
	Hi	776 (16.9)	293.7 (2.1)	100	0

[a]LOE -Loss of equilibrium
[b]NC – Not Conducted

2,4-D data summary:
- Mortality (5%-15%) and behavioral effects of white sturgeon observed with exposure to 2,4-D near or within maximum recommended application rate of 4 mg/L;
- No observed rainbow trout effects or mortality associated with 2,4-D exposure near or within maximum recommended application rate;
- 30 dps KRWS: LC50 calculated at 30.8 mg/L;
- 160 dps KRWS: LOEC observed at 38.9 mg/L based on loss of equilibrium; LC50>85.3 mg/L;
- 30 dps RBT: LC50 >387 mg/L and <776 mg/L;
- 160 dps RBT: LC50>294 mg/L; no significant mortality or loss of equilibrium occurred.

Summary of 2007 Laboratory Herbicide Toxicity Studies to Early Life Stage Juvenile White Sturgeon and Rainbow Trout

Of the three herbicides evaluated, our data indicate that triclopyr has the lowest probability of causing adverse effects to KRWS at the maximum recommended application rate (no observed effects up to 4.35 mg/L). Data from fluridone and 2,4-D toxicity tests show 5% KRWS mortality at concentrations less than the recommended application rates. Given that the KRWS is a critically endangered species, concerns exist regarding any level of mortality of these fish. Additional data addressing sub-lethal and lethal effects to KRWS from exposure to fluridone and 2,4-D within expected application rates and exposure times for anticipated field conditions are warranted prior to their use in the Kootenai River.

Acknowledgements:

Support for these studies was provided by the Kootenai Tribe of Idaho and Washington Department of Fish and Wildlife who provided hatchery white sturgeon eggs and fry for toxicity testing. Additional support was provided by Kurt Getsinger, U.S. Amy Corps of Engineers, for herbicide study design and data results review. Juvenile sturgeon illustration by Loucas Raptis.

References:

Dwyer, F.J., F.L. Mayer, L.C. Sappington, D.R. Buckler, C.M. Bridges, I.E. Greer, D.K. Hardesty, C.E. Henke, C.G. Ingersoll, J.L. Kunz, D.W. Whites, T. Augspurger, D.R. Mount, K. Hattala, and G.N. Neuderfer. 2005. Assessing Contaminants Sensitivity of Endangered and Threatened Aquatic Species: Acute Toxicity of Five Chemicals. Arch. Environ. Contam. Toxicol. 48:143-154.

Little, E.E. 2008a. Toxicity of chlorine to rainbow trout and white sturgeon from the Kootenai River and Columbia River. Abstract prepared for: Fish and Wildlife Service Spokane Field Office. Prepared by: Edward E. Little, Columbia Environmental Research Center, US Geological Survey, Columbia, MO. February 4.

Little, E.E. 2008b. Acute toxicity of copper to early life stage rainbow trout and white sturgeon from the Kootenai River and two life stages of white sturgeon from the Columbia River. Abstract prepared for: Fish and Wildlife Service Spokane Field Office. Prepared by: Edward E. Little, Columbia Environmental Research Center, US Geological Survey, Columbia, MO. February 4.

Little, E.E. 2008c. Acute toxicity of two herbicides, Renovate 3 and Sonar A.S., to two life stages of white sturgeon from the Kootenai River and to rainbow trout. Abstract prepared by: Edward E. Little, USGS Columbia Environmental Research Center. Submitted to: US Fish and Wildlife Service Region 1 Spokane Field Office. February 12.

Little, E.E. 2008d. Acute toxicity of the herbicide DMA 4 IVM to two life stages of white sturgeon from the Kootenai River and to rainbow trout. Abstract prepared by: Edward E. Little, USGS Columbia Environmental Research Center, Columbia, MO. Submitted to: FWS Region 1 Spokane Field Office. February 11.

APPENDIX B

PROJECT-SPECIFIC AMENDMENTS TO THE "UPPER COLUMBIA RIVER DRAFT GENERAL SITE HEALTH AND SAFETY PLAN FOR THE REMEDIAL INVESTIGATION AND FEASIBILITY STUDY"

Amendments to the "Upper Columbia River Draft General Site Health and Safety Plan for the Remedial Investigation and Feasibility Study" for:

Assessment of Surface Water Toxicity to White Sturgeon (*Acipenser transmontanus*) in the Upper Columbia River

Prepared by
ENTRIX, Inc.
Saskatoon, SK S7N 3B5, Canada

And

Environmental Toxicology Laboratory
Toxicology Centre,
University of Saskatchewan
44 Campus Drive
Saskatoon, SK S7N 3B5, Canada

Prepared for
Teck American Incorporated
P.O. Box 3087
Spokane, Washington 99220
USA

March 2009

Approval Page

Approved by: _____ Date: _____
[ENTRIX, Study Manager]

Approved by: _____ Date: _____
[U of S, Principal Investigator]

Approved by: _____ Date: _____
[U of S, Study Coordinator &
Co-Principal Investigator]

Approved by: _____ Date: _____
[U of S, Co-Principal Investigator]

Approved by: _____ Date: _____
[U of S, Co-Principal Investigator]

Approved by: _____ Date: _____
[Teck, Sponsor Project Manager]

SITE HEALTH AND SAFETY PLAN ACKNOWLEDGEMENT

Employee signature	**Affiliation**	**Date**
Employee signature	**Affiliation**	**Date**
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1.0 INTRODUCTION

This document serves as an amendment to the "Upper Columbia River Draft General Site Health and Safety Plan for the Remedial Investigation and Feasibility Study," hereinafter referred to as HASP, prepared by Integral Consulting Inc. and Parametrix in 2007. It provides additional information that is specific to the locations and the type of work associated with the "Assessment of Surface Water Toxicity to White Sturgeon (*Acipenser transmontanus*) in the Upper Columbia River." This document describes local and project-specific information and should be used in combination with the University of Saskatchewan (U of S) Environmental Toxicology Laboratory safety plan and all U of S Department of Health, Safety and Environment safety plans and directives. Finally, additional health and safety information is included in the individual standard operating procedures (SOPs; Appendix C).

Copies of both the HASP and this amendment must be in the custody of all employees working at the site during all times. All individuals performing work onsite must read, understand, and comply with the contents of both documents and their addenda before undertaking any work-related activities. Once the information has been read the individual must sign the "Site Health and Safety Plan Acknowledgement" forms provided both with the HASP and this amendment indicating that he/she understands these documents.

There may be modifications to this amendment during the conduct of the studies based on the judgment of the study team leaders, project manager, safety officer, Teck American Incorporated (Teck), or any of the liaisons on site (e.g., City of Trail, B.C.). Any changes will be presented to the onsite team as soon as possible during a safety briefing or other appropriate opportunities, and will be recorded in the study notebooks.

2.0 PROJECT MANAGEMENT AND OTHER KEY CONTACTS

Name	***Affiliation***	***Phone#***	***Cell#***	***Fax#***	***Comment***
Abenante, Larry	City of Trail	(250) 364-0825			Public Works Manager
Adzic, Marko	Teck, Spokane	(509) 892-2585	(509) 991-0842	(509) 459-4400	Teck Project Officer
Brown, Richard	Teck, Trail	(250) 364-4930			Power Coordinator
Duncan, Bill	Teck, Trail	(250) 364-4336	(250) 231-0234		Teck Project Liaison in Trail
Giesy, John	U of S	(306) 966-2096	(517) 614-6123	(306) 614-6123	U of S, Principal Investigator
Hecker, Markus	ENTRIX / U of S	(306) 966-5233	(517) 899-0594 or (306) 220-5757	(306) 966-4796	Project Manager; Liaison between Teck, U of S, and ENTRIX
Height, Dustin	City of Trail	(250) 368-3821	(250) 368-7880		Water Plant Operator; Liaison between Public Works, Senior Management and City Counsel
Hilts, Steven	Teck, Trail	(250) 364-4385	(250) 364-8269		Teck Site Issues
Impact Equipment (Harley)	Impact Equipment	(250) 364-9964	(250) 231-0552		Moving of Tanks and other Equipment
Janz, David	U of S	(306) 966-7434			U of S, Co-Principal Investigator
Liber, Karsten	U of S	(306) 966-7444			U of S, Co-Principal Investigator (Metal Analysis)
Teck Main Gate	Teck, Trail	(250) 364-8269			Teck Security
Tompsett, Amber	U of S		(306) 280-0275	(306) 966-4796	U of S Field Team Leader
U of S Field Phone	U of S		(306) 229-5234		Field Phone
Vardy, David	U of S		(306) 220-9825	(306) 966-4797	U of S Laboratory Team Leader

3.0 CHEMICAL HAZARD EVALUATION

In addition to the chemicals listed in the HASP, 10 percent buffered formalin will be used to preserve samples. The material safety data sheet for this chemical is provided in Section 5.0.

4.0 TRAINING AND HOSPITAL INFORMATION

4.1 Training

All personnel working on site and in the laboratory must complete the following University of Saskatchewan Department of Health, Safety and Environment classes, or equivalent courses:

- Laboratory Safety Course
- Biosafety Course
- Animal Use and Care Course

No hazardous waste operations (HAZWOPER) training is required because the above listed courses provide adequate training with regard to handling hazardous chemicals,

4.2 Hospital Information

Facility Name	***Hours of Operation***	***Phone #***	***Address***	***City***
Kootenay Boundary Regional Hospital/Trail regional Hospital	24h/7d	(250) 368-3311	1200 Hospital Bench	Trail
Castlegar & District Community Health Centre	8.00 – 16:30 (M – F)	(250) 365-7711	709 10th Street	Castlegar
Kootenay Lake Regional Hospital	8.00 – 16:30 (M – F)	(250) 352-3111	3 View Street	Nelson
Grand Forks Boundary Hospital	8.00 – 14.00 (M – F)	(250) 443-2100	7649 22nd Street RR 2	Grand Forks

5.0 MATERIAL SAFETY DATA SHEETS

Material Safety Data Sheet

Section 1. Product and Company Identification

Product Name	Buffered Neutral Formalin 10%	**Product Code**	R04586
Manufacturer	EMD Chemicals Inc. P.O. Box 70 480 Democrat Road Gibbstown, NJ 08027 Prior to January 1, 2003 EMD Chemicals Inc. was EM Industries, Inc. or EM Science, Division of EM Industries, Inc.	**Effective Date** **Print Date**	**3/27/2003** 5/3/2004

For More Information Call	**In Case of Emergency Call**
856-423-6300 Technical Service Monday-Friday: 8:00 AM - 5:00 PM	800-424-9300 CHEMTREC (USA) 613-996-6666 CANUTEC (Canada) 24 Hours/Day: 7 Days/Week

Synonym	None.
Material Uses	Laboratory Reagent
Chemical Family	Mixture.

Section 2. Composition and Information on Ingredients

Component	CAS #	% by Weight
FORMALDEHYDE	50-00-0	4
Methanol	67-56-1	<2
Sodium Phosphate, Dibasic, Anhydrous	7558-79-4	<0.7
Sodium Phosphate, Monobasic, Monohydrate	10049-21-5	<0.5
Water	7732-18-5	>92.8

+ Section 3. Hazards Identification

Physical State and Appearance	Liquid.
Emergency Overview	WARNING ! CANCER HAZARD CONTAINS MATERIAL WHICH CAN CAUSE CANCER HARMFUL IF SWALLOWED. CAUSES RESPIRATORY TRACT, EYE AND SKIN IRRITATION. MAY BE HARMFUL IF INHALED OR ABSORBED THROUGH SKIN. WARNING: This product contains a chemical(s) known to the State of California to cause cancer.
Routes of Entry	Absorbed through skin. Dermal contact. Eye contact. Inhalation. Ingestion.

Potential Acute Health Effects

Eyes	Hazardous in case of eye contact (irritant). Inflammation of the eye is characterized by redness, watering, and itching.
Skin	Hazardous in case of skin contact (irritant). Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. Non-permeator by skin.
Inhalation	Hazardous in case of inhalation (lung irritant). Non-hazardous in case of inhalation.
Ingestion	Hazardous in case of ingestion.

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Potential Chronic Health Effects

Carcinogenic Effects Classified + (Proven.) by OSHA [FORMALDEHYDE]. Classified A2 (Suspected for human.) by ACGIH, 2A (Probable for human.) by IARC [FORMALDEHYDE].

Additional information See Toxicological Information (section 11)

Medical Conditions Aggravated by Overexposure: Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4. First Aid Measures

Eye Contact	Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.
Skin Contact	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.
Inhalation	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Ingestion	If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

+ Section 5. Fire Fighting Measures

Flammability of the Product	Non-flammable.
Auto-ignition Temperature	Not applicable.
Flash Points	Not applicable.
Flammable Limits	Not applicable.
Products of Combustion	Not applicable.
Fire Hazards in Presence of Various Substances	Not applicable.
Explosion Hazards in Presence of Various Substances	**Risks of explosion of the product in presence of static discharge:** Slightly explosive in presence of open flames, sparks and static discharge. **Risks of explosion of the product in presence of mechanical impact:** Slightly explosive in presence of shocks.
Fire Fighting Media and Instructions	Not applicable.
Protective Clothing (Fire)	Not applicable.
Special Remarks on Fire Hazards	Not available.
Special Remarks on Explosion Hazards	Not available.

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+ Section 6. Accidental Release Measures

Small Spill and Leak Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

Large Spill and Leak Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Spill Kit Information The following EMD Chemicals Inc. SpillSolv (TM) absorbent is recommended for this product:
SX1340 Formaldehyde Treatment Kit

Section 7. Handling and Storage

Handling Avoid prolonged contact with eyes, skin, and clothing. Avoid contact with eyes. Do not ingest. Avoid breathing vapors or spray mists. Avoid prolonged or repeated contact with skin. Use only with adequate ventilation. Wash thoroughly after handling.

Storage Keep container tightly closed. Keep container in a cool, well-ventilated area.

+ Section 8. Exposure Controls/Personal Protection

Engineering Controls Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective occupational exposure limits.

Personal Protection

Eyes Splash goggles.

Body Lab coat.

Respiratory Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.

Hands Gloves.

Feet Not applicable.

Protective Clothing (Pictograms)

Personal Protection in Case of a Large Spill Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self-contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Product Name	Exposure Limits
FORMALDEHYDE	**EH40-MEL (United Kingdom (UK), 1997).** STEL: 2.5 mg/m^3 15 minute(s). STEL: 2 ppm 15 minute(s). TWA: 2.5 mg/m^3 8 hour(s). TWA: 2 ppm 8 hour(s). **ACGIH (United States, 2000).** CEIL: 0.37 mg/m^3 CEIL: 0.3 ppm **NIOSH REL (United States, 1994).** CEIL: 0.1 ppm 15 minute(s). TWA: 0.01 ppm 10 hour(s). **OSHA Final Rule (United States, 1989).** STEL: 2 ppm 15 minute(s). TWA: 0.75 ppm 8 hour(s). **OSHA Transitional Rule (United States, 1993).** STEL: 2 ppm 15 minute(s). TWA: 0.75 ppm 8 hour(s).

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Methanol	**ACGIH (United States, 1994). Skin** TWA: 262 mg/m³ STEL: 328 mg/m³ **OSHA (United States, 1989). Skin** TWA: 260 mg/m³ STEL: 325 mg/m³ **ACGIH (United States, 1994). Skin** STEL: 328 mg/m³ 15 minute(s). STEL: 250 ppm 15 minute(s). TWA: 262 mg/m³ 8 hour(s). TWA: 200 ppm 8 hour(s). **NIOSH REL (United States, 1994). Skin** STEL: 325 mg/m³ 15 minute(s). STEL: 250 ppm 15 minute(s). TWA: 260 mg/m³ 10 hour(s). TWA: 200 ppm 10 hour(s). **OSHA Final Rule (United States, 1989). Skin** STEL: 325 mg/m³ 15 minute(s). STEL: 250 ppm 15 minute(s). TWA: 260 mg/m³ 8 hour(s). TWA: 200 ppm 8 hour(s).
Sodium Phosphate, Dibasic, Anhydrous	Not available.
Sodium Phosphate, Monobasic, Monohydrate	Not available.
Water	Not available.

Section 9. Physical and Chemical Properties

Odor	Pungent.
Color	Clear. Colorless.
Physical State and Appearance	Liquid.
Molecular Weight	Not applicable.
Molecular Formula	Not applicable.
pH	7 [Neutral.]
Boiling/Condensation Point	The lowest known value is 64.55°C (148.2°F) (METHANOL). Weighted average: 99.08°C (210.3°F)
Melting/Freezing Point	May start to solidify at -0.1°C (31.8°F) based on data for: Water. Weighted average: -5.72°C (21.7°F)
Specific Gravity	Weighted average: 0.96 (Water = 1)
Vapor Pressure	The highest known value is 12.9 kPa (97 mmHg) (@ 20°C) (METHANOL).
Vapor Density	The highest known value is 1.11 (Air = 1) (METHANOL). Weighted average: 1.06 (Air = 1)
Volatility	99.9% (v/v). (METHANOL.)
Odor Threshold	The lowest known value is 0.05 ppm (FORMALDEHYDE) Weighted average: 33.14 ppm
Evaporation Rate	0.36 (Water) compared to (n-BUTYL ACETATE=1)
VOC	6 (%)
$LogK_{ow}$	Not available.
Solubility	Soluble in water.

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Section 10. Stability and Reactivity

Stability and Reactivity	The product is stable.
Conditions of Instability	Not available.
Incompatibility with Various Substances	Highly reactive with oxidizing agents, acids, alkalis. Slightly reactive to reactive with metals.
Rem/Incompatibility	Not available.
Hazardous Decomposition Products	COx , Na2O
Hazardous Polymerization	Will not occur.

Section 11. Toxicological Information

RTECS Number:	Formaldehyde	LP8925000
	Methanol	PC1400000
	Sodium Phosphate, Dibasic, Anhydrous	WC4500000
	Sodium dihydrogen phosphate monohydrate	Not available.
	Water	ZC0110000
Toxicity	Acute oral toxicity (LD_{50}): 42 mg/kg [Mouse]. (FORMALDEHYDE). Acute dermal toxicity (LD_{50}): 15800 mg/kg [Rabbit]. (METHANOL). Acute toxicity of the vapor (LC_{50}): 64000 ppm 4 hour(s) [Rat]. (METHANOL).	
Chronic Effects on Humans	**CARCINOGENIC EFFECTS**: Classified + (Proven.) by OSHA [FORMALDEHYDE]. Classified A2 (Suspected for human.) by ACGIH, 2A (Probable for human.) by IARC [FORMALDEHYDE].	
Acute Effects on Humans	Hazardous in case of eye contact (irritant). Inflammation of the eye is characterized by redness, watering, and itching. Hazardous in case of skin contact (irritant). Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. Non-permeator by skin. Hazardous in case of inhalation (lung irritant). Non-hazardous in case of inhalation. Hazardous in case of ingestion.	
Synergetic Products (Toxicologically)	Not available.	
Irritancy	Draize Test: Not available.	
Sensitization	Not available.	
Carcinogenic Effects	Classified + (Proven.) by OSHA [FORMALDEHYDE]. Classified A2 (Suspected for human.) by ACGIH, 2A (Probable for human.) by IARC [FORMALDEHYDE].	
Toxicity to Reproductive System	Not available.	
Teratogenic Effects	Not available.	
Mutagenic Effects	Not available.	

Section 12. Ecological Information

Ecotoxicity	Not available.
BOD5 and COD	Not available.
Toxicity of the Products of Biodegradation	The products of degradation are less toxic than the product itself.

Continued on Next Page

Buffered Neutral Formalin 10% **R04586** **Page: 5/7**

Section 10. Stability and Reactivity

Stability and Reactivity	The product is stable.
Conditions of Instability	Not available.
Incompatibility with Various Substances	Highly reactive with oxidizing agents, acids, alkalis. Slightly reactive to reactive with metals.
Rem/Incompatibility	Not available.
Hazardous Decomposition Products	COx , Na2O
Hazardous Polymerization	Will not occur.

Section 11. Toxicological Information

RTECS Number:	Formaldehyde	LP8925000
	Methanol	PC1400000
	Sodium Phosphate, Dibasic, Anhydrous	WC4500000
	Sodium dihydrogen phosphate monohydrate	Not available.
	Water	ZC0110000
Toxicity	Acute oral toxicity (LD_{50}): 42 mg/kg [Mouse]. (FORMALDEHYDE). Acute dermal toxicity (LD_{50}): 15800 mg/kg [Rabbit]. (METHANOL). Acute toxicity of the vapor (LC_{50}): 64000 ppm 4 hour(s) [Rat]. (METHANOL).	
Chronic Effects on Humans	**CARCINOGENIC EFFECTS**: Classified + (Proven.) by OSHA [FORMALDEHYDE]. Classified A2 (Suspected for human.) by ACGIH, 2A (Probable for human.) by IARC [FORMALDEHYDE].	
Acute Effects on Humans	Hazardous in case of eye contact (irritant). Inflammation of the eye is characterized by redness, watering, and itching. Hazardous in case of skin contact (irritant). Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. Non-permeator by skin. Hazardous in case of inhalation (lung irritant). Non-hazardous in case of inhalation. Hazardous in case of ingestion.	
Synergetic Products (Toxicologically)	Not available.	
Irritancy	Draize Test: Not available.	
Sensitization	Not available.	
Carcinogenic Effects	Classified + (Proven.) by OSHA [FORMALDEHYDE]. Classified A2 (Suspected for human.) by ACGIH, 2A (Probable for human.) by IARC [FORMALDEHYDE].	
Toxicity to Reproductive System	Not available.	
Teratogenic Effects	Not available.	
Mutagenic Effects	Not available.	

Section 12. Ecological Information

Ecotoxicity	Not available.
BOD5 and COD	Not available.
Toxicity of the Products of Biodegradation	The products of degradation are less toxic than the product itself.

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Buffered Neutral Formalin 10% **R04586** **Page: 6/7**

Section 13. Disposal Considerations

EPA Waste Number	U122 U154
Treatment	Incineration, fuels blending or recycle. Contact your local permitted waste disposal site (TSD) for permissible treatment sites. ALWAYS CONTACT PERMITTED WASTE DISPOSER (TSD) TO ASSURE COMPLIANCE WITH ALL CURRENT LOCAL, STATE AND FEDERAL REGULATIONS.

Section 14. Transport Information

DOT Classification	Not available.
TDG Classification	Not available.
IMO/IMDG Classification	Not available.
ICAO/IATA Classification	Not available.

Section 15. Regulatory Information

U.S. Federal Regulations	TSCA 8(b) inventory: FORMALDEHYDE ; Methanol; Sodium Phosphate, Dibasic, Anhydrous; Sodium Phosphate, Monobasic, Monohydrate; Water SARA 302/304/311/312 extremely hazardous substances: FORMALDEHYDE SARA 302/304 emergency planning and notification: FORMALDEHYDE SARA 302/304/311/312 hazardous chemicals: FORMALDEHYDE ; METHANOL; Sodium Phosphate, Dibasic, Anhydrous SARA 311/312 MSDS distribution - chemical inventory - hazard identification: FORMALDEHYDE : Fire Hazard, Immediate (Acute) Health Hazard, Delayed (Chronic) Health Hazard; METHANOL: Fire Hazard, Immediate (Acute) Health Hazard, Delayed (Chronic) Health Hazard; Sodium Phosphate, Dibasic, Anhydrous: Immediate (Acute) Health Hazard SARA 313 toxic chemical notification and release reporting: FORMALDEHYDE 4%; METHANOL 1.98% Clean Water Act (CWA) 307: No products were found. Clean Water Act (CWA) 311: FORMALDEHYDE ; Sodium Phosphate, Dibasic, Anhydrous Clean air act (CAA) 112 accidental release prevention: FORMALDEHYDE Clean air act (CAA) 112 regulated flammable substances: No products were found. Clean air act (CAA) 112 regulated toxic substances: FORMALDEHYDE
WHMIS (Canada)	Class D-2A: Material causing other toxic effects (VERY TOXIC). Class D-2B: Material causing other toxic effects (TOXIC). CEPA DSL: FORMALDEHYDE ; METHANOL; Sodium Phosphate, Dibasic, Anhydrous; Water This product has been classifed in accordance with the hazard criteria of the Controlled Product Regulations and the MSDS contains all required information.
International Regulations	
EINECS	FORMALDEHYDE 200-001-8 Methanol 200-659-6 Sodium Phosphate, Dibasic, Anhydrous 231-448-7 Sodium Phosphate, Monobasic, Monohydrate 231-449-2 Water 231-791-2
DSCL (EEC)	R22- Harmful if swallowed. R36/38- Irritating to eyes and skin.

Continued on Next Page

Buffered Neutral Formalin 10% **R04586** **Page: 7/7**

International Lists

Australia (NICNAS): FORMALDEHYDE ; Methanol; Sodium Phosphate, Dibasic, Anhydrous; Sodium Phosphate, Monobasic, Monohydrate; Water

Japan (MITI): FORMALDEHYDE ; Methanol; Sodium Phosphate, Dibasic, Anhydrous; Water

Japan (MOL): FORMALDEHYDE

Korea (TCCL): FORMALDEHYDE ; Methanol; Sodium Phosphate, Dibasic, Anhydrous; Water

Philippines (RA6969): FORMALDEHYDE ; Methanol; Sodium Phosphate, Dibasic, Anhydrous; Water

China: No products were found.

State Regulations

Pennsylvania RTK: FORMALDEHYDE : (special hazard, environmental hazard, generic environmental hazard); METHANOL: (environmental hazard, generic environmental hazard); Sodium Phosphate, Dibasic, Anhydrous: (environmental hazard, generic environmental hazard)
Massachusetts RTK: FORMALDEHYDE ; METHANOL; Sodium Phosphate, Dibasic, Anhydrous
New Jersey: Buffered Neutral Formalin 10%
California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer, birth defects or other reproductive harm, which would require a warning under the statute: FORMALDEHYDE
California prop. 65 (no significant risk level): FORMALDEHYDE
California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer which would require a warning under the statute: FORMALDEHYDE

Section 16. Other Information

National Fire Protection Association (U.S.A.)

Changed Since Last Revision +

Notice to Reader

The statements contained herein are based upon technical data that EMD Chemicals Inc. believes to be reliable, are offered for information purposes only and as a guide to the appropriate precautionary and emergency handling of the material by a properly trained person having the necessary technical skills. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use, storage and disposal of these materials and the safety and health of employees and customers and the protection of the environment. EMD CHEMICALS INC. MAKES NO REPRESENTATION OR WARRANTY OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE, WITH RESPECT TO THE INFORMATION HEREIN OR THE PRODUCT TO WHICH THE INFORMATION REFERS.

APPENDIX C

STANDARD OPERATING PROCEDURES

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Sample Management: Receiving, Preservation, Storage, Documentation, Decontamination, and Disposal

Version 1 May 11, 2008

Denise Kay, Markus Hecker and John P. Giesy

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 5206; 966-2096
Facsimile: (306) 966-4796

APPROVAL PAGE

Revisions to an existing SOP, addition of an SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By:	Denise Kay, Markus Hecker and John P. Giesy	Date: _____
Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

DEFINITIONS AND ACRONYMS

ETL	Environmental Toxicology Laboratory (University of Saskatchewan)
COC	Chain of Custody
CQA	Chemical Quality Assurance
LIMS	Laboratory Information Management System
UofS	University of Saskatchewan
DHSE	Department of Health, Safety and Environment (University of Saskatchewan)
PPE	Personal Protective Equipment
QA	Quality Assurance
SOP	Standard Operating Procedure
STS	Sample Tracking Sheet
SAP	Sampling and Analysis Plan

TABLE OF CONTENTS

1.0 PURPOSE

This standard operating procedure (SOP) specifies the requirements for sample receipt, control, record keeping, decontamination, and disposal at the Environmental Toxicology Laboratory at the University of Saskatchewan.

2.0 SCOPE AND APPLICATION

This SOP applies to the ETL for samples supplied from the Upper Columbia River White Sturgeon Studies.

3.0 SAFETY CONSIDERATIONS

Safety training and medical monitoring requirements are described in the Health and Safety Plan for the Upper Columbia River Remedial Investigation and Feasability Study.

In addition, there are various safety concerns regarding the receipt, storage, and disposal of sample containers at the ETL. Upon receipt, the sample containers will be monitored for breakage. If sample containers are broken, the appropriate personnel will be immediately notified and the Department of Health, Safety and Environment (DHSE) will be called in order to assess the hazard. DHSE will also be contacted in the case of chemical spills and will be responsible for the disposal of hazardous wastes.

3.1 Personal Protective Equipment

Personnel protective equipment (PPE), consisting of lab coats, safety glasses, and latex gloves will be worn at all times when handling samples.

3.2 Waste Management

All waste will be managed and disposed of in accordance with U of S-DHSE regulations. Waste management practices will include the control of all standards and solutions. This means that if required expired or used standards, associated solvents and other chemicals used for preservation and biological or element analysis will be disposed of in labeled waste containers and DHSE will be notified for waste pick up.

3.3 Sample Decontamination

If a spill occurs in the laboratory, DHSE will be notified immediately. The area where the spill occurred will be evacuated and marked.

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

The sample storage area is equipped with a locked freezer and a liquid nitrogen dewar in which samples are stored as appropriate. The freezer and dewar are connected to phone alarm systems that monitor temperature and notify laboratory personnel in cases of temperature and/or power related issues. A calibrated balance is also kept in the sample storage area and is used to weigh sub-samples.

No materials or reagents are used in sample receipt.

5.0 METHOD, PROCEDURES, AND REQUIREMENTS

5.1 Sample Receipt

The physical condition of coolers or other containers used for transportation, and each individual sample container will be inspected upon arrival at the ETL. The following objectives have been established for sample receiving:

A. Inspect sample coolers and samples for signs of damage upon receipt at the laboratory.

B. Attach air bill or shipping receipt to the chain of custody (COC) form.

C. Examine individual samples and record their status (frozen/ not frozen; immersed in preservation liquid, etc.) on a sample receipt form.

D. Verify that a COC form is submitted with samples, and that the COC contains all information required for analysis and reporting. Maintain custody of samples by ensuring that all dates, times, and signatures are provided on the COC forms.

E. Identify and reconcile any discrepancies between the COC and sample labels.

F. Verify that sample containers, labeling, or other requirements are correct. Assign a unique lab identification number to each sample and log samples into the sample tracking sheet (STS). (See attached STS.) Identify any hazards or special precautions associated with the incoming samples.

G. Notify appropriate laboratory and field study personnel when samples have arrived. These individuals are to be identified in either a Work Plan or SAP.

H. Track and document the handling of samples from receipt through data reporting to final disposal. This will be accomplished by keeping all of the log forms in a binder kept in the laboratory.

5.2 Sample Documentation

Upon arrival, the shipping receipts will be collected from the cooler and be stapled to the COC form. Samples submitted to the ETL will be accounted for by documenting their arrival and condition on COC and sample tracking sheets. Within the ETL, the STS will be used to monitor the samples whereabouts at all times. Aliquots removed will be recorded on the STS. While handling samples, any anomalies or problems will be noted in bound laboratory notebooks.

5.3 Sample Storage and Preservation

Samples will be stored in liquid nitrogen, freezer, fridge or locked storage room at room temperature (formalin preserved sturgeon samples) in the laboratory. This room is accessible only to lab personnel. The freezer and frideg will be set at -20° C and +4° C, respectively, and the temperature will be monitored daily. If for any reason there is a power outage or an increase in temperature, the facility manager on call and/or other lab personnel will be immediately notified by the automated phone alarm system that will automatically call the cell phone of the person on duty. The necessary action will then be taken to ensure that sample integrity is not compromised. If samples are removed from any of the storage compartments/units for any reason, this activity will be documented on the STS form. Copies of the forms will be placed in the records archive. When samples are removed for preparation and analysis, a sample extraction form will be completed.

5.3.1 Scheduled Monitoring

All dewars, refrigerators and freezers used in the ETL will be examined frequently due to constant use and will be monitored at a minimum daily. Freezer temperatures are maintained at a nominal -20° C. If the freezer temperature rises to -15° C, the liquid nitrogen levels decreases such that it triggers the alarm of the fridge temperature rises over +7°C corrective action must be taken. Actions include adjusting the thermostats, refilling liquid nitrogen, having the unit serviced, or moving the samples to another unit.

5.3.2 Sample Accountability

To ensure that all samples will be accounted for, the following guidelines will be followed:

A. The person obtaining the sample or submitting the sample to the laboratory for analysis must establish sample identity.

B. Integrity of sample must be maintained from collection to delivery.

C. Composition of sample must remain the same during handling and storage before analysis.

D. Evidence must exist of sample's receipt and COC record filled out, and appropriate personnel notified of the sample arrival.

E. Person preparing sample must not allow composition of sample to change or integrity to be questioned.

F. Analyst must ensure correct sample is analyzed.

G. Analyst must record all data contributing to the analysis.

H. Records must be kept to trace sample from retrieval through data reporting.

I. Special storage conditions must be documented.

5.3.3 Label and COC Discrepancies

Discrepancies between the sample labels and COC will be noted on the COC or Sample Receipt Form. The sample manager will resolve any documentation discrepancies by contacting the personnel that submitted samples. For discrepancies impacting sample viability (i.e., improper sample temperature) where a CAR is required to be completed, the sample manager will coordinate with the sample submitter, QA, and Project Study Group representatives to determine the appropriate corrective action.

6.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

The primary analyst shall document any anomalies and/or deviation from the specified method in a bound, serially numbered, laboratory notebook with tear-out carbon copies. All electronic files and hardcopies will be kept at the participating laboratory.

The carbon copies from data notebooks will be removed and archived in a separate building. Copies of the COC forms, the STS, and laboratory notes will be kept in 3-ring binders in separate places at all times in case of fire or other disaster.

7.0 RESPONSIBILITIES

Project Manager — Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principle investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

Principle Investigator — Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL) —David Vardy and Amber Tompsett, under the supervision of Markus Hecker, will oversee all research activities and supervise all personnel involved with the

assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager — Prof. Paul D. Jones will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

8.0 QUESTIONS OR COMMENTS

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

Jong Seong Khim, Ph.D.
jongseong.khim@usask.ca
(306) 966-5206

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

9.0 REFERENCES

Comprehensive Analytical Laboratory Services Quality Assurance Management Plan, April 1997.

Environmental Analytical Laboratory, Laboratory Quality Control Plan, April 1997.

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Maintenance of Sample Integrity, and Proper Usage of Refrigerators, Freezers, and Liquid Nitrogen Dewars

Version 1 May 11, 2008

Denise Kay, Markus Hecker and John P. Giesy

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 5206; 966-2096
Facsimile: (306) 966-4796

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Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

TABLE OF CONTENTS

1.0 PURPOSE

Guidelines have been established to ensure that refrigerators and freezers are used in a safe, clean, and efficient manner.

2.0 SCOPE AND APPLICATION

The following procedure outlines proper usage of refrigerators and freezers by the members of the Environmental Toxicology Laboratory (ETL), as well as sample logging and treating procedures.

This procedure describes guidelines for safe storage of samples and standards, sample storage locations, restrictions on the types of materials that may be stored in certain units, sample access, temperature monitoring, protection of sample integrity and alarm systems.

3.0 SAFETY CONSIDERATIONS

Safety training and medical monitoring requirements are described in the Health and Safety Plan for the Upper Columbia River Remedial Investigation and Feasability Study.

4.0 METHOD, PROCEDURES, AND REQUIREMENTS

4.1 Sample Storage Locations

A. Water samples are sometimes kept in short-term storage (24 to 48 hours) in the walk-in cooler #1, Room 118, Toxicology Building, University of Saskatchewan (U of S).

B. Feed, ingredients for feed, and substratum for invertebrate (artemia) culture are stored in the small Danby refrigerator located in Room 57, Toxicology Centre, U of S..

C. The walk-in freezer in Room 116 and the walk-in cooler in Room 118, Toxicology Building, U of S, are used to store tissue and whole organism samples from field and laboratory studies. The walk-in freezer is maintained at -20° C and the walk-in cooler is maintained at 4° C.

D. The Baxter Cryo-Fridge upright refrigerator/ freezer unit in Room 181, , Toxicology Building, U of S (Serial # Z19D-193854-ZD) is used for storage of samples to be analyzed. The refrigerated areas are maintained at 4° C, and the freezer areas at -20° C.

E. The Thermo Scientific Forma -86C Ultra Freezer (Serial # 812712-2490) in Room 261, Toxicology Building, U of S, is used to store biological samples for analysis. It is maintained at -80° C.

F. Samples for biochemical and molecular analyses are stored in the MVE Cryosystem 2000 liquid nitrogen (LN) dewar in Room 261, Toxicology Building, U of S.

4.2 Sample Access

All rooms and laboratories are locked at all times and are considered very secure. Each employee authorized to work there has a key to enter the rooms. The walk-in freezer and cooler are locked and access to these rooms is limited to ETL personnel.

4.3 Scheduled Monitoring

All refrigerators and freezers used by the Environmental Toxicology research group are examined frequently due to constant use and monitored weekly by reading the temperature from a thermometer located in each unit and recording the temperature in the Maintenance Log for Refrigerators, Freezers, and LN Dewars. Freezer temperatures are maintained at -20° C. If the freezer temperature rises to -15° C, the liquid nitrogen levels decreases such that it triggers the alarm of the fridge temperature rises over +7°C corrective action must be taken. Actions include adjusting the thermostats, refilling liquid nitrogen, having the unit serviced, or moving the samples to another unit.

Any incidents requiring corrective action are recorded in the Maintenance Log, a three-ring binder in the Biochemistry Lab, Toxicology Building, Room 261.

Maximum-minimum thermometers are located inside the walk-in cooler and inside the walk-in freezer. These thermometers are checked weekly and reset, and the maximum temperature for each unit is recorded in the logbook. Directions for use of the maximum-minimum thermometers are located in the Additional Notes section of the Maintenance Log binder.

4.4 Alarm System

The walk-in freezer and walk-in cooler can tolerate brief power outages or malfunctions without compromise of the samples they contain because of their large volume and large stored mass. However, it is important that any such malfunctions or power outages are recognized promptly; therefore, the walk-in freezer, walk-in cooler, Fisher Scientific Ultra Freezer and LN dewars are protected by temperature and power failure phone alarm systems. Instructions for these systems are posted with each unit and an additional emergency phone list is provided at the door of each laboratory/room. In case of a building power outage the freezers are all connected to the emergency generator. Be sure that the cause of the power outage is identified and corrected.

FREEZER ALARM

If the above alarms are triggered, it means that this freezer / refrigeration / dewar unit has a serious problem. Please take one of the actions listed below to save valuable research materials.

1. Consult the Laboratory On-Call List posted on the wall by the nearest telephone and call the responsible person.

2. Contact one of the following people:

    A. Jong Seong Khim at 966-5206 (office) or at 281-6204 (cell)

    B. Paul D. Jones at 966-5062 (office) of at 517-281-5666 (cell)

    C. Markus Hecker at 966-5233 (office) or 220-5757 (cell)

    D. Dr. John Giesy at 966-2096 (office) or at 517-614-6123 (cell))

    E. Any member of the ETL Management team at 966-4680

3. During evenings or weekends, if no one answers at the numbers above, call the campus operator and request physical plant emergency service for a freezer malfunction.

4. Report electrical and freezer malfunctions to the ETL manager Jong Seong Khim, Toxicology Building, room 135, or Shanda Sedgwick, Toxicology Building, room 125

Any Environmental Toxicology personnel who respond to an alarm will visually inspect the unit that gave the alarm and comment in the Maintenance Log binder. An estimate of the amount of time the unit was not functioning properly should be entered under Comments. If there is not enough space on the log sheet for a thorough description of the incident, refer the reader to the Additional Notes section and place a full report there. All electrical and freezer malfunctions should be reported to the ETL laboratory manager.

The walk-in freezer and walk-in cooler are connected to an audible alarm, phone alarm system and an auxiliary generator. If power from the main power grid is lost, the auxiliary generator automatically provides power and an alarm is called to U of S Public Safety. The alarm is then transferred to "on call" ETL personnel. There are two individuals on call at all times. These individuals can be contacted by telephone and carry pagers at all times. The Thermo Scientific Forma -86C Ultra Freezer in Room 261, also attached to the auxiliary power system and has an phone alarm system that in case of a power outage and/or temperature increase automatically calls the cell phones of ETL personnel on duty (a minimum of two persons are on duty at any

time). Otherwise, the procedures for this freezer are the same as those for the walk-in freezer, and records for its maintenance will be kept in the Maintenance Log in the Toxicology Building room 261.

The -196°C liquid nitrogen Dewar flasks are also equipped with audible alarms. If an alarm is registered, check the liquid nitrogen (N_2°; LN) level. If it is low, add liquid nitrogen from the upright LN tank in room 159 (chemistry laboratory) in the Toxicology Building. Then reset the alarm and record corrective actions in the Liquid Nitrogen section of the Maintenance Logbook in the Toxicology Building room 261. The liquid nitrogen Dewars will also be checked weekly for sufficient liquid nitrogen levels. See the section on procedures for and safe use of liquid nitrogen.

4.5 Guidelines for Proper use of Refrigerators / Freezers

A. All samples, standards, and reagents should be properly labeled with the identification of the substance in the container and the date. If appropriate, hazard warnings, concentration, and an expiration date should be added. All incoming chemicals should be labeled with the full name of the receiver and the date of receipt and should be logged into either a logbook or a database. Note: It is not acceptable to label only the rack holding many small tubes or vials.

B. Sample labels: All stored samples should be given new labels within 13 months past the date on the label. All samples must be in labeled sample boxes with a closing lid-preferably ECONSTOR 704 (Available from Fellowes Manufacturing Co., Perma Products, Atlanta, GA). Each individual sample in the box should be labeled with the following:

Project name:
Date collected:
Date placed in freezer/ cooler:
Sample type: (e.g. rainbow trout carcass)
Client name:
Client's sample ID:
Sample tracking # (Chain of Custody):

Samples should be labeled on the outside of the container or package as well as on a piece of paper placed inside the sample container. Labels should be written with pencil on paper. Do not use felt tip pens to write on glass or plastic. Use only permanent, waterproof markers to write on glass or plastic. For samples to be stored in liquid nitrogen see the Materials portion of the Liquid Nitrogen section.

C. Sample Box Labels: Samples should be grouped by project and/or sample type and placed in the storage boxes. Boxes should be labeled with the following information:

Project name:
Sample type:
Date placed in freezer:
Name of person who collected sample:
Client Name:
Location where sample was collected:
List of sample #s in box:

A sample log sheet should be filled out and a copy placed in both the sample box and the sample log binder. Note that there are numbers on the shelves in the walk-in freezer and cooler and that there is a map of the -80° C freezer in the sample log showing numbered storage areas. These numbers are the sample box location numbers. When a sample box is logged, the number corresponding to the area where it is stored should be recorded on the appropriate line in the logbook. This system makes the task of finding a sample box much simpler and minimizes the amount of time that the deep freeze door is open. An entry should be made in the Maintenance Log every time the freezer is entered so that in case of a malfunction, it can be determined when the unit was last known to be functioning properly.

D. Samples and analytical standards should be kept in separate refrigerators or freezers.

E. Food or beverages for human consumption should never be stored in a refrigerator or freezer where standards, samples, or reagents are stored.

F. Buffers such as TRIS, HEPES, and phosphate buffers should be kept for no longer than one month. The pH of a buffer should be checked regularly at the temperature at which the buffer is intended to function. Buffers with sucrose should be filtered before storage; filtering will increase the storage life to no longer than two weeks. Buffers should be marked with an expiration date and disposed of after that date.

G. Samples and standards should be kept in containers that will prevent them from spilling or otherwise contaminating other stored materials. Items easily tipped or without tight lids should be placed inside other containers to prevent spilling. For example, a flask with a Parafilm cover might be placed inside a wide beaker.

H. Each person using storage space in a refrigerator or freezer should check routinely (once a month) for old buffers, glassware, etc., that he/she has left behind and should remove unneeded items.

I. For reasons of cleanliness, safety, and limited space, each person using storage space in a refrigerator or freezer may remove any items not in compliance with the above guidelines to a designated area in another refrigerator or freezer. Every reasonable

effort must then be made to contact the person responsible, including contact by mail and telephone, and a note describing the item(s) removed and the new storage location should be posted on the refrigerator or freezer in which the items were found. If the items are not claimed and dealt with properly, they will be destroyed one month after removal.

4.6 Safety and Usage of Liquid Nitrogen Dewars

(Taken from the CRC Handbook of Laboratory Safety)

A. Flammability: Liquid nitrogen, liquid helium, and metal surfaces made very cold by liquefied gases can condense oxygen from the atmosphere, causing oxygen to build up or become entrapped in enclosed spaces. This greatly increases the risk of fire, and may cause even non-combustible materials like carbon steel to burn under the right conditions. Make sure that the area where liquid nitrogen Dewars are stored is well ventilated.

B. High Pressure: Liquefied nitrogen is stored at or near its boiling point, so that some gas is always present in the container that holds it. Be aware that liquid nitrogen expands rapidly when allowed to warm up, so make certain that containers that hold it include an allowance for the gaseous phase.

C. Materials: Materials that are otherwise pliable or tough may become brittle and shatter under the extreme cold temperatures of liquid nitrogen. Materials suitable for cryogenic temperatures include Dacron, Teflon, Kel-F, asbestos impregnated with Teflon, Mylar, Nylon, stainless steel (300 series), copper, bronze, aluminum, and brass. Important: Do not use glass vials to store samples in a liquid nitrogen Dewar. The glass will shatter. Use only plastic Cryovials with tamper-proof vinyl stick-on labels. Also, do not use wooden materials with liquid nitrogen, since wood (or asphalt) saturated with oxygen might explode when subjected to mechanical shock. If in doubt, consult a materials manual or call DHSE to make certain that liquid nitrogen will not cause a problem with the materials with which it is to be used.

D. Personnel:

1. Avoid hazards of fire, high pressure, and material failures listed above.

2. Even very brief contact of body parts with fluids or materials at cryogenic temperatures can cause burns similar to thermal burns. Prolonged contact will cause exposed parts to freeze and become brittle. The eyes are particularly sensitive to this type of trauma, so always wear eye protection while working with liquid nitrogen.

3. While liquid nitrogen is not itself toxic, it can cause asphyxiation by displacing air, so store and use liquid nitrogen only in well-ventilated areas.

4. Equipment should be kept very clean to avoid dangerous contamination of liquid nitrogen stores.

5. When there is a possibility of personal contact with liquid nitrogen, wear full-face protection, an impervious apron or lab coat, cuffless trousers, and closed shoes (no sandals). Do not wear jewelry. Gloves may or may not be worn. If worn, gloves should be impervious and loose fitting so that they can be easily thrown off the hand if liquid nitrogen is spilled inside them. Potholder type protection for the hands is probably best. Do not touch the interior of the Dewar or anything that has been recently removed from it without protection for the hands.

6. Do not tilt a Dewar flask to pour out the liquid, as this may damage the container.

E. Contamination: Oxygen can build up in liquid nitrogen containers if the cap is not kept on or if the entire volume of liquid in the container is not occasionally replaced, i.e., the Dewar is continually refilled from larger containers without ever allowing it to become totally empty, increasing the chances of contamination with oxygen. If the liquid takes on a bluish color, it is contaminated with oxygen and should be treated as a dangerous, potentially explosive material.

5.0 RESPONSIBILTIES

Project Manager — Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principle investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

Principle Investigator — Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

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6.0 QUESTIONS OR COMMENTS

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Markus Hecker
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

Jong Seong Khim, Ph.D.
jongseong.khim@usask.ca
(306) 966-5206

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

7.0 REFERENCES

Good Laboratory Practice Standards. 40 CFR Part 160. Environmental Protection Agency, 1989.

Steere, Norman V. CRC Handbook of Laboratory Safety. Chemical Rubber Co., Cleveland, 1967. pp 314-323.

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

MAINTENANCE OF SAMPLE CHAIN-OF-CUSTODY

Version 1 May 30, 2007

Paul D. Jones, Jong Seong Khim, and John P. Giesy

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 5206; 966-2096
Facsimile: (306) 966-4796

APPROVAL PAGE

Revisions to an existing SOP, addition of an SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By:	Paul D. Jones, Jong Seong Khim, and John P. Giesy	Date: 05/30/07
Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

CHAIN OF SAMPLE CUSTODY

To maintain the chain of custody, samples must be treated in the manner described below.

1. Samples are inspected upon arrival to see if they are in agreement with any accompanying inventory of samples. The sample condition should be noted and compared to expected condition.

2. Samples are assigned laboratory identification and custodian. Obtain documents of shipment or transfer from incoming person (e.g., bill of lading number or mail receipt).

3. Chain of Custody Record should be maintained throughout the project. The custodian has the responsibility to maintain the integrity of the sample. The custodian must maintain the original Chain of Custody Record. A copy may be obtained for the relinquishing party, if so desired.

4. Samples should be stored in refrigerators or freezers to maintain stability. The refrigerators and freezers are located in the Wet Chemistry Laboratory (Room 158) and Biochemistry Laboratory (Room 261).

SAMPLE SECURITY

Sample storage locations

1. Samples for analysis are stored in various refrigerators within the Wet Chemistry Laboratory.
2. Post analysis storage is in walk-in freezer (Room 116).

Sample access

1. Wet Chemistry Laboratory. The Wet Chemistry Laboratory is a locked laboratory within a locked building and is considered secure during working and non-working hours. All Environmental Toxicology Laboratory (ETL) employees have access to the freezer and refrigerators.

2. Walk-in freezer (Room 116). Access is limited to authorized personnel.

SCHEDULED MONITORING

All refrigerators and freezers used by the ETL are examined frequently due to constant use and monitored weekly by reading and recording the temperature on the Freezer/Refrigerator Temperature Record Sheet from a thermometer located in each unit. Freezer temperatures are maintained at a nominal -20^{o} C. If the temperature rises to -15^{o} C, corrective action must be taken. Actions include adjusting thermostats, having the unit serviced, or moving the samples to another unit.

1. During working hours, contact Dr. Paul D. Jones at 5062, or anyone else in the ETL who will help determine what should be done.

2. During evenings and weekends, if it isn't simply a matter of a freezer door being left open, call the campus operator and request physical plant emergency service for a freezer malfunction and Dr. Paul D. Jones at 281-2996 or 517-281-5666.

SAMPLE ACCOUNTABILITY

1. Must be a representative portion of product sampled.

2. Identity of sample must be established by person obtaining sample or submitting the sample to the laboratory for analysis.

3. Integrity of sample must be maintained from collection to delivery.

4. Composition and integrity of sample must remain the same during handling and storage before analysis.

5. Evidence must exist of sample's receipt and a Chain of Custody Record filled out and appropriate personnel notified of the sample arrival.

6. Person preparing sample must not allow composition of sample to change or integrity to be questioned.

7. Analyst must ensure the correct sample is analyzed.

8. Analyst must record all data contributing to analysis.

9. Records must be kept to trace sample from time obtained through reporting, including storage.

10. Special storage conditions must be documented

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

STANDARD OPERATING PROCEDURE FOR WEIGHING USING ANALYTICAL BALANCES

Version 1 May 11, 2008

Markus Hecker and John P. Giesy

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

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Authored By: Markus Hecker and John P. Giesy Date: _____

Supervisor Review By: _____ Date: _____

Reviewed By: _____ Date: _____
(QA Coordinator)

WEIGHING TECHNIQUES

1. Check level and cleanliness of balance.

2. Balances must not be affected by room drafts; close balance doors.

3. Always weigh into containers or use weigh papers. Under no circumstances should excess standard or reagent be returned to its original container.

4. Weigh quickly. It is better to accurately weigh close to a desired weight in one or two steps than to repeatedly manipulate a sample to obtain a round number. For example 10.02 and 9.81 are just as good weights as 10.00.

5. Objects weighed must be at room temperature.

6. Choose a balance appropriate for the mass to be weighed. For example, a 4-decimal place balance is need for a 0.0100 g standard whereas a 40.00 g soil sample requires only a 2-place balance.

7. At the beginning of each phase of the study the balance must be checked using standard reference weights and the results recorded on the Analytical Balance Maintenance Calibration Record form.

8. Routine and preventative maintenance is purchased from the balance manufacturer at recommended intervals, nominally once in two years.

Environmental Toxicology Laboratory
Toxicology Research Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Equipment Maintenance and Calibration

Version 1, May 11, 2008

Amber Tompsett, David Vardi, Markus Hecker and John P. Giesy, Ph.D.

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 966-2096
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Authored By:	Amber Tompsett, David Vardi, Markus Hecker, and John P. Giesy	Date: 05/11/2008
Reviewed By: (Project Manager/ Principal Investigator)	_____	Date: _____
Reviewed By: (Study Director)	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____
Reviewed By: (Sponsor)	_____	Date: _____

DEFINITIONS AND ACRONYMS

ETL Environmental Toxicology Laboratory, Toxicology Centre (University of Saskatchewan)

UCR Upper Columbia River

U of S University of Saskatchewan

TABLE OF CONTENTS

1 PURPOSE

The purpose of this SOP is to provide guidelines for the maintenance and calibration of equipment used in the UCR white sturgeon studies conducted by the ETL.

2 SCOPE AND APPLICATION

This SOP will be applied to all equipment used in the UCR white sturgeon studies including multiparameter meters, pipettors, and balances.

3 SAFETY CONSIDERATIONS

There are no safety issues for this SOP.

4 EQUIPMENT, MATERIALS, AND REAGENTS

The equipment used in UCR white sturgeon studies will be:

- Sartorius Balance (Mettler Toledo, Inc, serial number 37030129)
- Ohaus Adventurer Pro Balances (VWR, Cat. #11379-144)
- Certified balance weights (Troemner Inc., serial number 13637)
- VWR sympHony Multiparameter Research Meters (VWR, Cat #11388-328)
- Pipetman Pipettors: P100, P200, P1000, P5000

5 METHOD, PROCEDURES, AND REQUIREMENTS

This SOP will provide details on the methods used to calibrate and maintain equipment used in the UCR white sturgeon experiments. In cases the calibration procedures are identical to the manufacturer's descriptions, it will be referred to these and the according document will be attached to this SOP as an APPENDIX.

5.1 Equipment Calibration:

5.1.1 Balances (Sartorius and Ohaus)

The balances will be calibrated before each use. They will be calibrated using check weights provided by the manufacturer. The weight of the check weights will be recorded in a balance log along with the date of use, user's name, and whether the balance recorded the check weights within 10% (APPENDIX I).

5.1.2 VWR sympHony Multiparameter Research Meters

pH probe
The pH meter will undergo a two point calibration before each use. Two standard buffer solutions are recommended for precise calibration. The first (near the electrode isopotential point (pH 7.0) and the second near the expected sample pH (pH 4.0 or 10.0). Calibration will be recorded on a data sheet once it is performed (APPENDIX II).

Conductivity probe
The conductivity meter will undergo a two point calibration before each use. Standard buffer solutions will be used. Calibration will be recorded on a data sheet once it is performed (APPENDIX II).

Dissolved oxygen (DO) Probe
No user calibration is required (APPENDIX II).

Temperature probe
No user calibration is required (APPENDIX II).

5.1.3 Pipetman pipettors

Pipettors will be calibrated on a weekly basis. Each pipettor will be used to draw up a volume of liquid that will then be weighed to confirm that the volume is correct. The serial number of each pipettor will be recorded along with the date, presumed volume, weight of the liquid, calibrator's name and whether the pipettor was adjusted will be recorded on a log sheet (APPENDIX III).

5.2 Equipment Maintenance:

5.2.1 Balances

The balances will be brushed off after each use, and kept covered between uses.

5.2.2 VWR sympHony Multiparameter Research Meters

Between measurements, the probes will be rinsed with distilled water. The filling hole cover will be removed when taking measurements, but will be put back in place during

storage. The pH probe will be kept in buffer solution of 3M KCl in between uses. All other probes will be stored according to manufacturer's protocol (APPENDIX II).

6 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

All maintenance and calibration procedures shall be entered in the appropriate note books and instrument logs. Instrument logs are kept with each piece of equipment, and a copy of all files is kept with Shanda Sedgwick in room 125 at the Toxicology Building.

7 RESPONSIBILITIES

Project Manager — Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principle investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

Principle Investigator — Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL) —David Vardy and Amber Tompsett, under the supervision of Markus Hecker, will oversee all research activities and supervise all personnel involved with the assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager — Prof. Paul D. Jones will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

8 QUESTIONS OR COMMENTS

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker Paul D. Jones, Ph.D.

mhecker@entrix.com
(306) 966-5233

paul.jones@usask.ca
(306) 966-5062

Jong Seong Khim, Ph.D.
jongseong.khim@usask.ca
(306) 966-5206

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

Environmental Toxicology Laboratory
Toxicology Research Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Experimental Exposure Systems for Field- and Laboratory-Based Studies of Aquatic Organisms Under Fluvial Conditions

Version 1, May 1st, 2008

David Vardy, B.S., Amber Tompsett, M.S., Markus Hecker, Ph.D.,
And, John P. Giesy, Ph.D.

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
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Authored By:	David Vardy, Amber Tompsett, Markus Hecker, and John P. Giesy	Date: 05/1/2008
Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

DEFINITIONS AND ACRONYMS

ETL	Environmental Toxicology Laboratory, Toxicology Centre (University of Saskatchewan)
DQO	Data Quality Objective
DHSE	Department of Health Safety and Environment
F	Female
FTxFT	Female Thread by Female Thread
ID	Inner Diameter
M	Male
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
SxM	Socket by Male
SOP	Standard Operating Procedure
UCR	Upper Columbia River
U of S	University of Saskatchewan

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

This document describes the design, construction, maintenance and utilization of artificial flow-through exposure systems to be used in *in situ* and laboratory studies under simulated fluvial conditions. These portable, self-contained exposure systems can be set up directly in the field or in the laboratory to be used with a water source of choice (e.g. river-water, laboratory water, etc.). The flow regime in the re-circulating exposure units can be adjusted such that it simulates fluvial conditions of interest (e.g. the flow-regime can be adjusted such that it accommodates the specific requirements for different life-stages or riverine fish such as sturgeon). The purpose of this document is to describe the methods to assemble exposure system structures, where to obtain the parts and how to arrange the experimental setup.

2.0 SUMMARY OF METHOD

The method described herein covers the initial construction and assemblage of all materials required for a fully functioning exposure system as well as considerations to be taken when choosing an experimental layout. Details of all building materials and suppliers are provided. A list of supplier contact information is included at the end of the SOP.

3.0 SAFETY CONSIDERATIONS

All safety considerations will be in accordance with U of S DHSE procedures and with the requirements of the U of S ETL Safety Manual. These requirements include:

1. All persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students);
2. All persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee.

Other job specific safety training will be provided by qualified members of the ETL.

Specific Safety Concerns

1. Personnel will be working in close proximity to water and electricity, such that electrocution is an imminent danger. For this reason, it is important to assure that all equipment is certified for this use and to always wire equipment and use ground fault interruption (GFI) circuits at all times. Do not stand in water while touching wiring, extension chords, or electrically powered equipment.
2. The exposure system frames and wet tables are heavy structures and care should be taken when lifting and assembling.
3. All chemicals and flammable liquids that are to be used should be disposed of in properly labeled waste containers and stored in appropriate storage bins.

4.0 EQUIPMENT AND MATERIALS

4.1 Flow-through exposure chambers

- Artificial flow-through exposure chambers and screen dividers are constructed from ¼" plexi-glass and are heat welded with special glue. The material and structures are supplied and fabricated by WD Plastics (Saskatoon SK). Each exposure chamber contains 7 removable screen dividers for controlling flow regime.
- ¼" puck board reservoir baffles and exposure chamber dividers are also supplied and fabricated by WD Plastics.

4.2 Incubation/hatching jars

- Mini, egg hatching jars are supplied by Aquatic Ecosystems (Apopka. FL; Cat. #J32).

4.3 Wet table

- The fiberglass wet tables are open 4' x 4' boxes, 8" deep. A 2" FTxFT bulk head fitting is installed at the bottom of one end for a stand pipe. The wet table, with installed bulk head, is fabricated by Progressive Yard Works Ltd (Saskatoon, SK).

4.4 Exposure system table frame

- The steel table frame is supplied and fabricated by Elance, steel fabricating Co. Ltd (Saskatoon, SK). Each exposure system table frame consist of a 4' ¼" x 4' ¼" welded L-bracket top frame, four 30" removable steel legs, four 4" steel bolts and four steel nuts.

4.5 Reservoir and mixing tank

- 85L polyethylene reservoir tanks (part # 70394-0) and 1000L polyethylene oval vertical mixing tanks with 8" lids and 1" FTxFT bulkheads (part # 60014-1) are supplied by Quality Molded Plastics Ltd (Saskatoon, SK).

4.6 Pumps

- Pulsafeeder pulsatron diaphragm metering pumps (model # LEH7SAPHC3-XXX) are supplied by Viking Pump of Canada Inc. and magnetic drive march pumps (part # W30HD) are supplied by Aquatic Eco-systems Inc, Saskatoon SK.

4.7 Chillers

- Water chillers are supplied by Aqua Logic Inc (Apopka, FL).

4.8 Plumbing equipment

- Bulkhead fittings are supplied by Western/Westlund. Ball valves, adapters, bushings, tees, PVC piping, and clamps are supplied by Aquifer Distribution Ltd (Saskatoon, SK). Some plumbing equipment can also be found at Home Depot. Quick coupler cam locks are supplied by Green Line Hose and Fittings (Saskatoon, SK), and hose/tubing is supplied by Goodall Rubber Company of Canada (Saskatoon, SK).

4.9 Tools and miscellaneous equipment

- Tools such as drills, drill bits, Teflon tape, and saws can be found at Home Depot. Miscellaneous equipment such as PVC/ABS cement, silicon, fiberglass mosquito netting, etc., can also be found at Home Depot or Canadian Tire.

5.0 METHODS AND PROCEDURES

5.1 Overview

A holding tank contains the water/solution mixture of interest to test in the experimental exposure system. The mixture is delivered from the tank to the 85L exposure system reservoir via a metering pump. The mixture then travels from the reservoir to the exposure system treatment chambers via a re-circulating march pump. The mixture flows from one end of the treatment chamber to the other and exits through a drain hole, which in turn is connected back to the 85L reservoir. There is an overflow drain out the back of each reservoir to discard wastewater and a baffle to prevent short-circuiting of the inflow to the overflow drain (Fig 1). The test solution mixture may be cooled to the desired temperature by either placing a chiller unit inside the 85L reservoir or by re-circulating chilled water through the wet table.

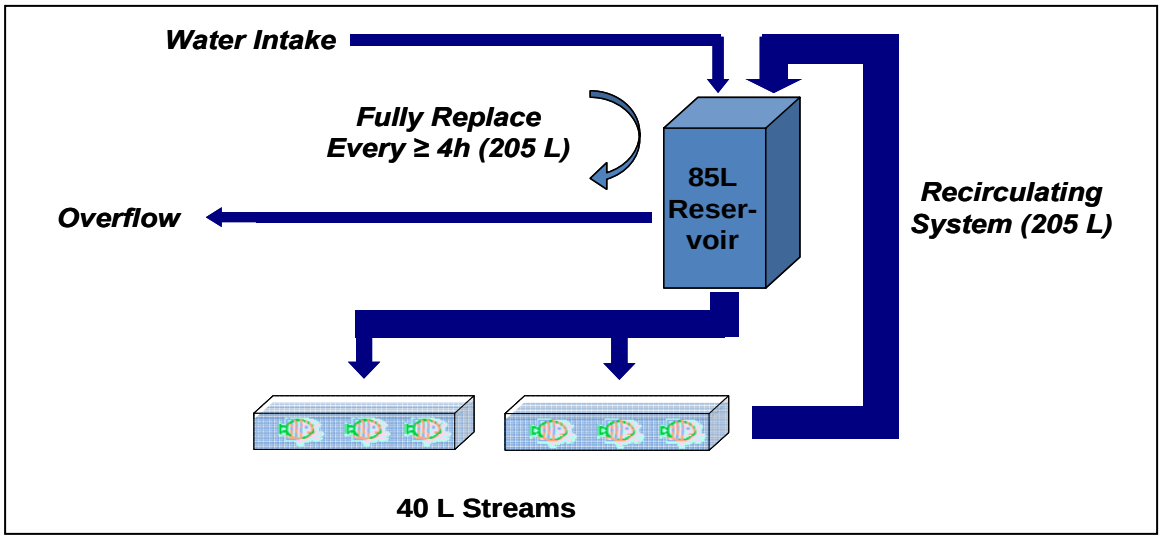

Figure 1. Experimental exposure system. Each true replicate exposure system consists of one 85L reservoir and two or three 40L chambers.

5.2 Experimental Setup

- All piping threads should be wrapped in Teflon tape prior to attachment.
- All PVC/ABS piping joints should be glued with the appropriate cement.

5.2.1 Exposure system table frames

- Arrange the experimental exposure systems in such a fashion that enables easy access, preferably from all sides.
- Assemble the steel table frame. Attach 4 steel legs to the steel top frame by sliding each leg into a sleeve on the frame and secure with a bold and nut.

5.2.2 Wet table

- A fiberglass wet table is placed inside the table frame.
- If using a water bath to cool the treatment chambers:
    - Fabricate a 6” standpipe from 2” diameter PVC piping
    - Attach the wet table standpipes into the bulkhead fitting of the wet table using a 2” SxM adapter.
    - Attach a drain hose to the bulkhead fitting on the underside of the wet table using a 2” PVC M adapter and run the drain hose back to the chilled water supply.
    - Attach a delivery hose from the chilled water supply to the wet table frame using PVC piping and a clamp. Attach a ball valve at the end so that the water flow can be adjusted.

5.2.3 Treatment chambers and incubation/hatching jars

- Each wet table can hold up to 6 plexiglass treatment chambers side by side.
- Attach a 1” FTxFT bulkhead to the 2” hole at the end part of each chamber.
- Each treatment chamber has 6 removable screen inserts to control the flow regime. Silicon fiberglass mosquito netting over the rectangular holes on the inserts in order to reduce water flow and to prevent the fish from passing through.
- Egg hatching jars are placed in the font end of the exposure chambers. The first screen at the front end of the chamber is removed and the test solution is pumped through the top of the standpipe of the hatching jar (from the reservoir) when the experiment is initiated (Fig 2.)

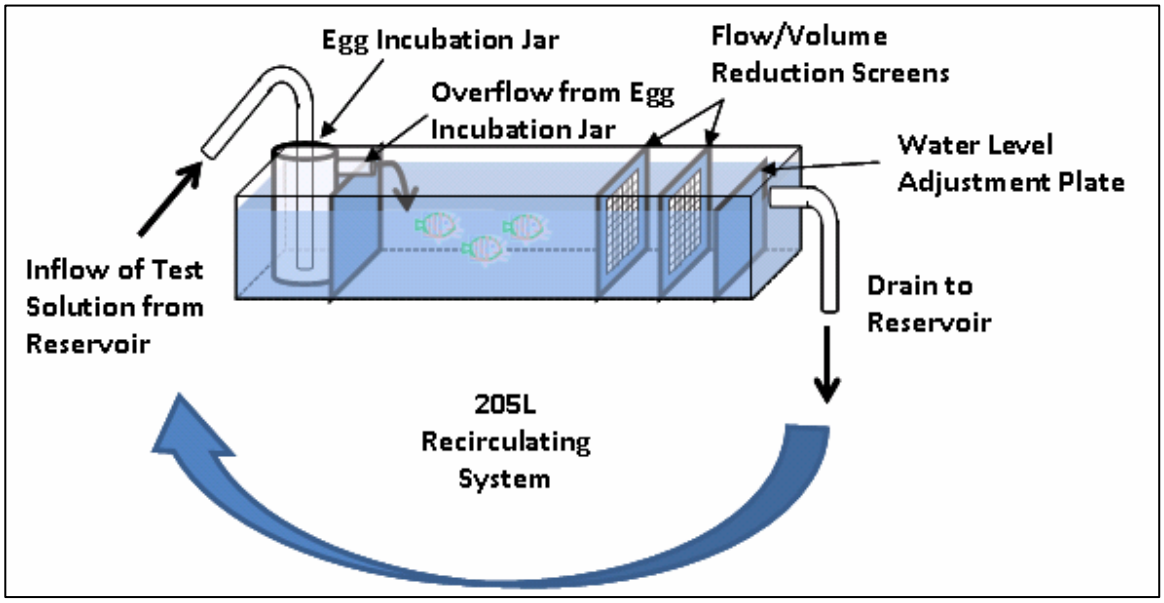

Figure 2. Exposure Chamber. This schematic represents one exposure chamber with egg hatching jar.

- A right angle 1" M PVC adapter is attached to the exterior side of the bulkhead fitting at the end of the exposure chambers.
- Tubing is attached to the adapters.
- Depending on the number of pseudo-replicates per table, combine the drain pipe tubing from common doses into one hose with an appropriate splitter.
- Run one drain hose (per true replicate) from each splitter back into the appropriate reservoir.
- Attach a right angle ½" PVC nozzle to the front of each treatment chamber using a clamp.
- Place a 48" x 24" x ¼" puck board divider between each true replicate on the table.

5.2.4 Reservoirs

- Reservoirs and lids must be cut to the appropriate size to fit under the table frame.
- Drill a 2" output hole in the center of one face of the reservoir near the bottom so that it will align with the march pumps. Attach a FTxFT 1" bulkhead. Attach a 1" ball valve and a M cam lock to the bulkhead.
- Drill a 2" intake hole in the center of the reservoir on the same face as the previous hole near the top (about 3" below the lid). Attach a FTxFT 1" bulkhead. Attach a M cam lock to the bulkhead.

- Drill a 2” overflow hole to the far side, near the top, in the reservoir face directly opposite the face in which the two previous holes were drilled. **Make sure the center of this hole is at least 1” lower then the previously drilled intake hole**. This hole is the overflow hole and must be lower than the previously drilled intake hole. Attach a FTxFT 1” bulkhead.
- Drill a 4” hole in the center of the bottom of the 23” x 8” x ¼” puck board baffle. Silicon this baffle, with the hole on the bottom, to the inside corner nearest the overflow hole of the reservoir. Make sure the baffle will separate the intake water from the overflow water.
- Drill a 2” hole in the corner of the reservoir lid opposite the reservoir overflow hole. Attach a FTxFT 1” bulkhead and a 1” M PVC adapter.
- Place the reservoirs under the exposure system frame and wet table.
- Attach black flexible sump-pump hose to the overflow drain at the back of the reservoir to discard of the wastewater.
- Attach the drain hose from each splitter of the exposure chambers to the 1” M PVC adapter in the lid of the appropriate reservoir.

5.2.5 March pump and manifold

- Attach a F cam lock to the intake of the march pump. Connect the march pump intake to the M cam lock at the bottom output of the reservoir.
- Attach a 4-way or 3-way splitter, depending on the number of pseudo-replicate exposure chambers per true replicate being used, to the output of the march pump. This is the initial piece of the manifold that will allow for water flow regulation to each exposure chamber.
- Attach two ball valves in series to each output arm of the splitter (this will allow the water flow to be shut off without disrupting the flow settings).
- Attach M PVC adapters and tubing to the second ball valve of each of the manifold arms and run the tubing to the right angle ½” PVC nozzle that was previously attached to the front of each treatment chamber.

5.2.6 Metering pumps

- Place the metering pumps next to their corresponding reservoirs.
- Attach a 3/8” ID hose barb to a F cam lock and attach to the M intake cam lock at the top of the reservoir.
- Attach 3/8” ID tubing from the hose barb to the output of the corresponding metering pump.

5.2.7 Mixing tanks and receiving tanks

- Position the mixing tanks (used in static renewal of test solution) or the receiving tank (used in continuous delivery of test solution from river or lake source) in such a way that they are easily accessible and so that they are as close as possible to their corresponding exposure systems.
- Attach a 3-way splitter to the 1" bulkhead of the tank.
- Attach ball valves to each of the output arms of the splitter.
- Attach a 3/8" ID hose barb to the ball valves on the splitter.
- Attach 3/8" ID tubing from the intake of the metering pumps to the 3/8" ID hose barb on the splitter on the corresponding tank.

5.3 Pump Settings and Flow Rate

5.3.1 Metering pump

Using the current pumping system the turnover rate of the water/mixture in the 205L re-circulating system can be varied from one to four times per 24hr period. This is controlled by the settings on the metering pump.

Metering pump settings and adjustments:

- Disconnect the cam lock on the reservoir intake at the top of the reservoir.
- Position the hose so that it is discharging into a graduated cylinder.
- Time the discharge for 60 seconds using a stopwatch and a measuring cylinder (or for 15 secs and multiply by 4).

- Regulate the discharge rate (ml/min) by adjusting the frequency and stroke knobs on the metering pump and repeat timing of discharge until the desired flow is achieved:
    - Set Stroke rate to nearest value of flow (Table 1.). Use Stroke Length to calibrate fine adjustment to achieve desired rate. Stroke length should be kept as close to 100% as possible for maximum efficiency.

Table 1. Stroke rate adjustments for metering pump.

Stroke rate	10	15	20	30	40	50	60	70	80	90	100
mL/min	110	140	210	330	400	480	580	695	755	800	900
L/day	160	200	300	475	575	690	835	1000	1090	1150	1300

- Ensure that the hose is re-connected once the flow rate is achieved.

5.3.2 March pump and manifold

The recirculation rate of the water/mixture from the reservoir to the treatment chambers can be varied from approximately 2L/min to 15L/min by adjusting the ball valves on the manifold attached to the march pump. Never completely shut off the ball valves on the manifold as the back pressure may damage the pump.

Manifold settings:

- Place a beaker under the right angle ½" PVC nozzle in the front of each treatment chamber corresponding to the manifold of interest.
- Fully open the ball valves on the manifold.
- Start the march pump and measure the flow rate into the beakers. Adjust the second ball valves on the manifold arms until the flow into each exposure chamber is at the desired rate.
- If the flow ever needs to be shut off for an exposure chamber, adjust the first ball valves on the manifold so that the set flow rate remains the same once normal flow is restored. This is will prevent having to re-calibrate the manifold.

6.0 TAKE-DOWN OF EXPOSURE SYSTEM

Ideally, all cleaning and acid washing should be done as soon as possible once the experiment is terminated. All tanks, streams and wet tables must be scrubbed clean before algae hardens and dries. **Aquarium/ wet lab appropriate soap should be used for cleaning.**

- Mixing tanks should be scrubbed using soap and water to remove any algal/fungal buildup and then acid washed (5% HCl) for a minimum of 8 hours.
- Exposure chambers can be cleaned first by scrubbing with water and soap and then with an acid bath (5% HCl). The plexiglass should be subjected to the acid bath for a minimum of 8 hours, and then well rinsed with clean water.
- Reservoirs can be cleaned first by scrubbing with water and soap and then with an acid bath (5% HCl). The plastic should be subjected to the acid bath for a minimum of 8 hours, and then well rinsed with clean water.
- To clean the March pump, run clean water through it to dilute any contaminants. **Note:** Do **not** use acid as it will corrode the metal parts of the pump.
- To clean the metering pump, run clean water through it to dilute any contaminants. If needed, remove the four screws to disassemble the head assembly. Check the injection valve assembly, the discharge valve cartridge and the suction valve cartridge - these may have lime or other substances blocking

them or preventing the ball from functioning properly. **Note:** Do **not** use acid as it will corrode the metal parts of the pump.

- Clean the area of concern with a small brush, being careful not to scratch or damage the apparatus.

7.0 MANUFACTURERS' CONTACT INFORMATION

WD Plastics
826- 56th St. East
Saskatoon, SK, S7K 5Y8
(PH) 306-934-6844 (FAX) 306-934-6842

Elance Steel Fabricating Company Ltd.
40 Unger St. North Corman Park
Site 404, Box 3, RR #4
Saskatoon, SK, S7K 3J7
(PH) 306-931-4412 (FAX) 306-931-7683

Progressive Yard Works Ltd.
3423 Millar Ave.
Saskatoon, SK, S7K 6J4
(PH) 306-244-6911 (FAX) 306-244-6913

Aquatic Eco-Systems, Inc.
2395 Apopka Blvd.
Apopka, FL, 32703
Orders and General Inquiries: 877-347-4788
Free Tech Support: 407-598-1401
Fax: 407-886-6787

Aquifer Distribution Ltd.
227A Venture Crescent
Saskatoon, SK, S7K 6N8
(PH) 306-242-1567 (FAX) 306-665-2115

Quality Molded Plastics Ltd.
Site 412, Box 280, RR#4
71st. St. Rd. & Highway 16W
Saskatoon, SK, S7K 3J7
(PH) 306-242-4494 (FAX) 306-242-4122

Goodall Rubber Co. of Canada
2902 Miners Ave Saskatoon, SK, S7K 4Z7
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8912-60 Avenue Edmonton, AB T6E 6A6
1-888-VIK-PUMP (845-7867)
(FAX) 780-466-9131
For parts ordering 519-256-5438

8.0 RECORDS, DOCUMENTATION AND QC REQUIREMENTS

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

9.0 RESPONSIBILITIES

Project Manager — Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principle investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

Principle Investigator — Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL) —David Vardy and Amber Tompsett, under the supervision of Markus Hecker, will oversee all research activities and supervise all personnel involved with the assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager — Prof. Paul D. Jones will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

10.0 QUESTIONS OR COMMENTS

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

Jong Seong Khim, Ph.D.
jongseong.khim@usask.ca
(306) 966-5206

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

Environmental Toxicology Laboratory
Toxicology Research Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Care and Maintenance of Early White Sturgeon (*Acipenser transmontanus*) Life-Stages under Laboratory Conditions

Version 1, April 25, 2008

Amber Tompsett, M.S., David Vardy, B.S., Markus Hecker, Ph.D.,
and John P. Giesy, Ph.D.

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 966-2096
Facsimile: (306) 966-4796

APPROVAL PAGE

Revisions to an existing SOP, addition of a SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By: Amber Tompsett, David Vardy, Markus Hecker, and John P. Giesy Date: 4/25/2007

Supervisor Review By: _____ Date: _____

Reviewed By: _____ Date: _____
(QA Coordinator)

DEFINITIONS AND ACRONYMS

ETL	Environmental Toxicology Laboratory, Toxicology Centre (University of Saskatchewan)
DQO	Data Quality Objective
DHSE	Department of Health Safety and Environment
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
UCR	Upper Columbia River
U of S	University of Saskatchewan

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

The purpose of this document is to describe methods necessary to culture and maintain White Sturgeon from fertilized eggs obtained from wild adult fish in the laboratory, including maintenance of cultures in field-based mobile laboratories. This document describes culture requirements for eggs (2-3 days post-fertilization) through larvae up to 90 d post-hatch.

2.0 SUMMARY OF METHOD

The method described herein covers the maintenance of proper conditions for early life-stage White Sturgeon cultures. This includes instructions for feeding the fish, cleaning tanks, maintaining water quality, and routine monitoring procedures to ensure health and growth of larvae and juveniles.

3.0 SAFETY CONSIDERATIONS

All safety considerations will be in accordance with U of S-DHSE procedures and with the requirements of the U of S ETL Safety Manual. These requirements include: all persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students); all persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee. Other job specific safety training will be provided by qualified members of the ETL.

Specific Safety Concerns

Personnel will be working in close proximity to water and electricity, such that electrocution is an imminent danger. For this reason, it is important to assure that all equipment is certified for this use and to always wire equipment and use ground fault interruption (GFI) circuits at all times. Do not stand in water while touching wiring, extension chords, or electrically powered equipment.

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

4.1 Flow-through chamber set up

- Artificial flow-through chamber system (set up as described in SOP-ETL6032) or similar flow-through system fed by suitable water source

4.2 Daily maintenance of fertilized White Sturgeon eggs

- Egg hatching jars (Aquatic Ecosystems, Cat. #J32)

4.3 Daily maintenance of larval White Sturgeon

- Bloodworms; Species: *Chironomus plumosus* (Hagen, Cat #A-9558)
- Mortar and pestle
- *Artemia* Cysts; Species: *Artemia salina* (Aquatic Ecosystems, Cat #BS90)
- Marine salt (Aquatic Ecosystems, Cat #SC1431)
- Separatory funnel (VWR, Cat #4300-1000)
- Air pumps and hoses
- Nets (Aquatic Ecosystems, Cat #AN4 & AN6)
- Net soak (Aquatic Ecosystems, Cat #NS8)
- Iodine (Aquatic Ecosystems, Cat #ID10)
- Buckets (Canadian Tire)

5.0 METHODS, PROCEDURES AND REQUIREMENTS

5.1 Flow-through chamber set up

- Flow-through chamber systems should be fabricated as outlined in SOP-ETL6032.
- Flow-through chambers are fed by a suitable water source (i.e. laboratory water, river water, exposure water).
- Flow-through chambers should be allowed to run for 3-4 days before eggs or larvae are added to the chambers.
- If the experiment will begin from the egg stage, an egg hatching jar should be placed into one exposure chamber in each re-circulating system when flow-through chambers are set up.

5.2 Daily maintenance of fertilized White Sturgeon eggs

1. Eggs are obtained 1-2 days post-fertilization from the Kootenay Trout Hatchery in late June/early July.
2. Eggs are transported to the appropriate location (i.e. ETL at U of S, UCR field sites) via motor vehicle or airplane in plastic bags filled with pure oxygen. Bags are to be sealed after filling with oxygen. Transport time should not exceed 24h.
3. When bags are opened, they should be aerated and kept at a constant temperature to avoid mass mortality.
4. Eggs are then randomly divided into the egg hatching jars in the mesocosms. Each jar should hold approximately $\geq$ 900 eggs.

5. The flow of water through the flow-through chambers should be adjusted to properly circulate and suspend the white sturgeon eggs in the hatching jars.
6. The eggs should be kept in suspension until hatch (~15 days post-fertilization).
7. Water quality measurements should be performed daily and water samples taken as outlined in SOP-ETL2096.
8. Cleaning of the mesocosms and egg hatching jars should be performed as needed with designated brushes soaked in net soak or iodine solution.
9. Dead eggs should be removed at least twice a day and processed as outlined in SOP-ETL4071.

5.3 Daily maintenance of larval White Sturgeon

1. When white sturgeon eggs hatch, they should be removed from the egg hatching jars and evenly randomly allocated into the exposure chambers in the corresponding re-circulating system. Egg hatching jars should then be thoroughly cleaned, disinfected in iodine, and stored.
2. Sturgeon larvae should be observed daily. Once the first individuals reject their "yolk-plugs" (black plug that can be observed at the rear of yolk sack) as observable by black plugs laying on the bottom of the exposure chambers, the sturgeon should be fed.
3. Small (up to 5cm) white sturgeon larvae should be fed 4 times daily as follows:
    - 8-9AM: each stream should be fed 5mL bloodworms that have been crushed with a mortar and pestle to a smooth puree. Bloodworms are spread on the feeding strips and placed into the streams.
    - 12PM: each stream is fed freshly hatched *Artemia* (*culture outlined below). Evenly split the *Artemia* hatched from 1L of culture.
    - 4-5PM: feed each stream 5mL bloodworms (as above)
    - 8-9PM: feed each stream 5mL bloodworms (as above)

    ***Note:** To culture artemia, mix 1L of fresh water with 1 tablespoon of marine salts and 1 tablespoon of artemia cysts in a plastic separatory funnel. Aerate with an air hose and small aquarium pump and incubate in a 25-30C water bath for 12-24 hr.
3. Larger (more than 5cm) white sturgeon should be fed 3-4 times daily as follows:
    - 8-9AM: feed each stream 10mL crushed bloodworms

- 12PM: feed each stream freshly hatched artemia. Evenly split 2L of culture.
- 4-5PM: feed each stream 10mL crushed bloodworms
- 8-9PM: feed each stream 10mL crushed bloodworms
- Depending upon fish condition, the 4-5PM and 8-9PM feedings may be combined into one feeding of 10mL of crushed bloodworms at 6-7PM.

4. Water quality measurements should be taken and water samples preserved as outlined in SOP-ETL2096.
5. Flow-through chambers should be cleaned as needed with designated brushes disinfected in net soak or iodine.
6. Dead white sturgeon should be removed and processed as outlined in SOP-ETL4071.

6.0 RECORDS, DOCUMENTATION AND QC REQUIREMENTS

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

7.0 RESPONSIBILITIES

Project Manager — Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principle investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

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Study Team Leaders (STL) —Amber Tompsett and David Vardy under the supervision of Markus Hecker will oversee all research activities and supervise all personnel involved with the culture and maintenance of early white sturgeon life-stages. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

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Environmental Toxicology Laboratory
Toxicology Research Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Preparation and preservation of White Sturgeon (*Acipenser transmontanus*) eggs and larvae for histology, molecular biology endpoints, metal analysis, and evaluation of nutritional state

Version 1, April 27, 2008

Amber Tompsett, M.S., David Vardy, B.S., Markus Hecker, Ph.D.,
and John P. Giesy, Ph.D.

Supported through:
Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
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44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
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Phone: (306) 966-5062; 966-2096
Facsimile: (306) 966-4796

APPROVAL PAGE

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Authored By:	Amber Tompsett, David Vardy, Markus Hecker, and John P. Giesy	Date: 04/28/2008
Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

DEFINITIONS AND ACRONYMS

ETL	Environmental Toxicology Laboratory, Toxicology Centre (University of Saskatchewan)
DQO	Data Quality Objective
DHSE	Department of Health Safety and Environment
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
UCR	Upper Columbia River
U of S	University of Saskatchewan

TABLE OF CONTENTS

1 SCOPE AND APPLICATION

The purpose of this document is to describe procedures for preserving White Sturgeon eggs and larvae for histological analysis, molecular biology analysis, metals analysis, and nutritional analysis over the course of White Sturgeon exposures. Methods are described for preparing daily mortalities, samples taken at points during the course of the experiment, and samples taken at exposure termination.

2 SUMMARY OF METHOD

The information provided herein is intended to provide the information required to properly prepare and preserve White Sturgeon samples for various forms of subsequent laboratory analysis.

3 SAFETY CONSIDERATIONS

All safety considerations will be in accordance with U of S-DHSE procedures and with the requirements of the U of S ETL Safety Manual. These requirements include: all persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students); all persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee. Other job specific safety training will be provided by qualified members of the ETL.

Specific Safety Warnings:

1. Formalin is a dangerous chemical. It is a human carcinogen and can cause damage to skin and eyes. Contact with formalin should be avoided at all times. Because formalin is volatile, avoid breathing the fumes. Always use gloves and use in a well ventilated area, outside or in a hood when working in the laboratory.
2. Liquid nitrogen is extremely cold and can cause frost bite. Avoid contact with the skin. Always wear protective clothing, such as gloves. Liquid nitrogen can splash and freeze tissue on contact. Thus it is a hazard to eyes. Therefore, always wear full eye protection (goggles) when using liquid nitrogen.

4 EQUIPMENT, MATERIALS, AND REAGENTS

4.1 Preparation and preservation of daily mortalities

- Nets (Aquatic Ecosystems, Cat #AN4 & AN6)
- Net soak (Aquatic Ecosystems, Cat #NS8)
- Iodine (Aquatic Ecosystems, Cat #ID10)
- Buckets (Canadian Tire)

- Paper towel
- Calipers (Canadian Tire)
- Forceps (VWR, Cat #25715-043)
- Balance (VWR, Cat #11379-144)
- 20mL sample vials (VWR, Cat #66021-679)
- 10% formalin (VWR, Cat #VW3239-7)
- 70% ethanol

4.2 Preparation and preservation of time point samples

- Nets (Aquatic Ecosystems, Cat #AN4 & AN6)
- Net soak (Aquatic Ecosystems, Cat #NS8)
- Iodine (Aquatic Ecosystems, Cat #ID10)
- Buckets (Canadian Tire)
- MS-222 (Sigma, Cat #A5040)
- Paper towel
- Calipers (Canadian Tire)
- Forceps (VWR, Cat #25715-043)
- Balance (VWR, Cat #11379-144)
- Scissors (VWR, Cat #25870-002)
- 7mL sample vials (VWR, Cat #66022-387)
- 10% formalin (VWR, Cat #VW3239-7)
- 70% ethanol
- Cryovials (VWR, Cat #16001-102)
- Cryovial storage racks (VWR, Cat #16001-166)
- Liquid nitrogen
- Dewar
- -20C freezer

4.3 Preparation and preservation of samples at exposure termination

- Same supplies needed as for time point samples

5 METHODS, PROCEDURES, AND REQUIREMENTS

5.1 Preparation and preservation of daily mortalities (performed twice daily)

1. Remove dead eggs or larvae from a single exposure chamber with a disinfected and rinsed net.
2. Place samples on a piece of paper towel to blot excess water.

3. Egg samples can be pooled and weighed on the balance down to the nearest 0.001 g, then preserved in 10% formalin for 24 h in a properly labeled 20mL sample vial.
4. Larvae samples are individually measured with the calipers down to the nearest 0.01 mm.
5. Larvae samples are then pooled and weighed on the balance, then preserved in 10% formalin for 24 h in a properly labeled 20mL sample vial.
6. Very large larvae can be individually weighed and placed into an individual properly labeled 7 mL sample vial.
7. All weight and length values should be recorded on the appropriate data sheets.
8. After 24 h the formalin in the vials is replaced with 70% ethanol for long-term storage until histological analysis can be completed. CAUTION: Ethanol is extremely flammable!
9. Samples can be stored at room temperature.
10. Sample transfers must be completed using proper chain of custody procedures (see SOP-ETL4006)

5.2 Preparation and preservation of time point samples

1. White sturgeon (WS) larvae will be sampled from each exposure chamber at multiple points during the course of experiments.
2. WS larvae should be removed from chambers with cleaned and rinsed nets.
3. WS larvae are then placed into a 1g/L solution of MS-222 until euthanized.
4. WS larvae are removed from MS-222 and blotted dry on paper towels.
5. All samples should be individually weighed on the balance and measured with calipers.
6. Weight and length data is recorded on the appropriate data sheets.
7. Histology samples:
    - Open the body cavity of large larvae with the dissecting scissors.
    - Place the larvae into a properly labeled 7mL sample vial that has been filled with 10% formalin.
    - Allow sample to fix for 24 hr.
    - Replace formalin with 70% ethanol for long-term storage.

- Samples can be stored at room temperature.

8. Molecular biology, nutritional status, and metal residue samples:
   - Place WS larvae samples into individual properly labeled cryovials.
   - Flash freeze vials in liquid nitrogen in a dewar.
   - Samples can be placed into cryovial racks before freezing if the dewar is equipped with canes (field dewars are not). If not, samples should be transferred to a dewar with canes and racks for long-term storage.
9. Sample transfers must be completed using proper chain of custody procedures (see SOP-ETL4006).

5.3 Preparation and preservation of samples at exposure termination

1. At exposure terminations, all white sturgeon larvae in all streams will be sampled.
2. Follow the guidelines outlined in 5.2 of this document for processing and preserving samples for histology, molecular biology, metals analysis, and nutritional analysis.

6 RECORDS, DOCUMENTATION AND QC REQUIREMENTS

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

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will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

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Environmental Toxicology Laboratory
Toxicology Research Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Measurement of Water Quality and Processing of Water Samples during White Sturgeon (*Acipenser transmontanus*) Exposures

Version 1, April 27, 2008

Amber Tompsett, M.S., David Vardy, B.S., Markus Hecker, Ph.D.,
and John P. Giesy, Ph.D.

Supported through:
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Authored By:	Amber Tompsett, David Vardy, Markus Hecker, and John P. Giesy	Date: 04/28/2008
Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

DEFINITIONS AND ACRONYMS

ETL	Environmental Toxicology Laboratory, Toxicology Centre (University of Saskatchewan)
COC	Chain of Custody
DOC	Dissolved organic carbon
DQO	Data Quality Objective
DHSE	Department of Health Safety and Environment
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
TOC	Total organic carbon
UCR	Upper Columbia River
U of S	University of Saskatchewan
WQ	Water Quality

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1.0 SCOPE AND APPLICATION

The purpose of this document is to describe methods necessary to make water quality measurements on exposure chambers and process water samples during White Sturgeon exposures, both in the U of S laboratory and in the mobile laboratory.

2.0 SUMMARY OF METHOD

The method described herein describes the appropriate methods for taking daily water quality measurements from exposure chambers during White Sturgeon exposures. In addition, methods for processing periodic water samples for extended analysis are described.

3.0 SAFETY CONSIDERATIONS

All safety considerations will be in accordance with U of S-DHSE procedures and with the requirements of the U of S ETL Safety Manual. These requirements include: all persons involved in research at the ETL shall complete basic laboratory safety, new employee orientation, bio-safety, and radiation safety courses offered by the DHSE (summer student orientation will suffice for summer students); all persons involved in research using vertebrate subjects must have taken proper training through the Animal Use and Care Committee. Other job specific safety training will be provided by qualified members of the ETL.

Specific Safety Concerns

Personnel will be working in close proximity to water and electricity, such that electrocution is an imminent danger. For this reason, it is important to assure that all equipment is certified for this use and to always wire equipment and use ground fault interruption (GFI) circuits at all times. Do not stand in water while touching wiring, extension chords, or electrically powered equipment.

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

4.1 Daily water quality measurements

- Ammonia nitrogen kit (LaMotte, Cat #7674)
- Nitrate nitrogen kit (LaMotte, Cat #7418-01)
- Hardness kit (LaMotte, Cat #4482-DR-LI)
- Multi-function meter for pH, temperature, dissolved oxygen, and conductivity (VWR, Cat #11388-328)

4.2 Periodic water quality measurements

- Chlorine kit (VWR, Cat #66170-136)
- Phosphate kit (VWR, Cat #66121-568)
- Sulfate kit (VWR, Cat #66121-530)

4.3 Preparation of water samples for metal analysis and detailed water quality analysis

- Water filtration apparatus (VWR, Cat #28199-406)
- 0.47µm water filters (VWR, Cat #28157-960)
- Plastic forceps (VWR, Cat #83009-010)
- Ultrapure nitric acid (VWR, Cat #CANX0408-7)
- Water sample bottles (VWR, Cat #EP156-125WMN)
- 1mL pipetter
- 1mL pipette tips
- Water bath (plastic tote will work)
- Ultra-pure water
- Hydrochloric acid
- Small Petri dishes (VWR, Cat #25384-332)
- -20C freezer

4.4 Preparation of water samples for TOC and DOC analysis

- Water filtration apparatus (VWR, Cat #28199-406)
- 0.47µm water filters (VWR, Cat #28157-960)
- Water sample bottles (VWR, Cat #EP156-125WMN)
- Hydrochloric acid
- Small Petri dishes (VWR, Cat #25384-332)
- -20C freezer

5.0 METHODS, PROCEDURES, AND REQUIREMENTS

5.1 Daily water quality measurements

1. WQ measurements should be made from one exposure chamber in each recirculating system every day. Chambers should be alternated so that WQ measurments are made on each different chamber over 2-3 days.
2. UCR field exposures: Make measurements for ammonia nitrogen, nitrate nitrogen, and water hardness using the kits according to the manufacturer's protocol. Copies of kit instructions are available in SOP notebooks and as electronic copies from UCR team members.

U of S laboratory exposures: Make kit measurements for those parameters that are not performed by the Liber lab. Copies of kit instructions are available in SOP notebooks and as electronic copies from UCR team members.

3. Using the multi-parameter water quality monitor, make measurements for temperature, pH, dissolved oxygen and conductivity.
4. Record all WQ measurements on the appropriate data sheet(s) following QA/QC requirements.
5. Any abnormal WQ measurments, especially high temperatures or ammonia levels, should be immediately reported to a team leader.

5.2 Periodic water quality measurements

1. WQ kits to make measurements for chlorine, phosphate, and sulfate are available to be used at important time points or when needed.
2. These measurements should be made in one exposure chamber per recirculating system the first day water is supplied to the mesocosms, before eggs/larvae are added to chambers, after eggs/larvae are added to chambers, once per week during the exposures in the field, at exposure termination, and whenever UCR team members deem necessary.
3. Record all WQ measurements on the appropriate data sheet(s) following QA/QC requirements.
4. Any abnormal WQ measurements shold be immediately reported to a team leader.

5.3 Preparation of water samples for metal analysis and detailed water quality analysis

1. Water samples should be prepared as outlined in the sampling plan provided by the UCR team leaders.
2. Place water filtration units, water filters, water sample tranfer bottles and plastic tweezers into a 5% hydrochloric acid bath 12-24 hrs before the samples will be taken.
3. Rinse the water filtration units, filters, bottles, and tweezers in an Ultrapure water bath.
4. Remove a 150mL water sample from one exposure chamber in each exposure treatment (i.e. each river water treatment, each metal dose, controls) with a water sample transfer bottle.

5. Assemble the water filtration units with water filters in place.
6. Place the water samples into the filtration units.
7. Allow samples an appropriate amount of time to pass through filters.
8. Disassemble filtration units and decant filtered water samples into properly labeled 125mL sample bottles. Fill bottles to approximately 1 inch from top. Discard excess filtered sample.
9. Acidify filtered samples with 1mL of Ultrapure nitric acid.
10. Cap bottles.
11. Place filters into properly labeled individual Petri dishes.
12. Freeze filtered water and filter samples at -20C until analysis by Liber lab. (Field samples will be shipped to U of S periodically for analysis by Liber lab.)
13. Record samples taken on the proper data sheet(s) and in the laboratory notebook.
14. All sample transfers, both between laboratories and from the field, should be performed with proper chain of custody documentation. See SOP-ETL4006 for COC details.

5.4 Preparation of water samples for TOC and DOC analysis

1. Water samples should be prepared as outlined in the sampling plan provided by the UCR team leaders.
2. Place water filtration units, water filters, water sample tranfer bottles and plastic tweezers into a 5% hydrochloric acid bath 12-24 hrs before the samples will be taken.
3. Rinse the water filtration units, filters, bottles, and tweezers in an Ultrapure water bath.
4. Remove a 150mL water sample from one exposure chamber in each exposure treatment (i.e. each river water treatment, each metal dose, controls) with a water sample transfer bottle.
5. Assemble the water filtration units with water filters in place.
6. Place the water samples into the filtration units.
7. Allow samples an appropriate amount of time to pass through filters.
8. Disassemble filtration units and decant filtered water samples into properly labeled 125mL sample bottles. Fill bottles to approximately 1 inch from top and cap. Discard excess filtered sample. The filtered sample contains the DOC content.

9. Place the filter into a properly labeled Petri dish. The filter contains the undissolved portion of the carbon content.
10. Preserve the water samples for analysis by acidifying the samples with 1mL of hydrocholoric acid.
11. Freeze water samples and filters at -20C.
12. The water sample represents DOC content; filter + water sample is TOC. Carbon analysis will be performed by .
13. Record samples taken on the proper data sheet(s) and in the laboratory notebook.
14. All sample transfers, both between laboratories and from the field, should be performed with proper chain of custody documentation. See SOP-ETL4006 for COC details

6.0 RECORDS, DOCUMENTATION AND QC REQUIREMENTS

All procedures, activities, anomalies and/or deviation from the specified method shall be recorded in a bound, serially numbered, laboratory notebook. In case of routine procedures that are identical to those conducted at an earlier time, the earlier description can be referred to without writing the complete procedures down again by indicating procedure, notebook page, date and initial.

7.0 RESPONSIBILITIES

Project Manager — Dr. Markus Hecker will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principle investigator and other U of S personnel, Teck Cominco personnel, and the sponsor Project Manager.

Principle Investigator — Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL) —Amber Tompsett and David Vardy under the supervision of Markus Hecker will oversee all research activities and supervise all personnel involved with the culture and maintenance of early white sturgeon life-stages. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager — Prof. Paul D. Jones will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

8.0 QUESTIONS OR COMMENTS

Please feel free to contact the following persons with any questions, comments, etc., you may regarding the procedures outlined in this SOP.

Markus Hecker, Ph.D.
mhecker@entrix.com
(306) 966-5233

Paul D. Jones, Ph.D.
paul.jones@usask.ca
(306) 966-5062

Jong Seong Khim, Ph.D.
jongseong.khim@usask.ca
(306) 966-5206

John P. Giesy, Ph.D.
john.giesy@usask.ca
(306) 966-2096

Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Data Package Review

Alan Blankenship, Markus Hecker and John P. Giesy

Toxicology Centre and
Department of Veterinary Biomedical Sciences

Correspondence to:
Environmental Toxicology Laboratory
Toxicology Centre
44 Campus Drive,
Saskatoon, Saskatchewan, S7N 5B3
Canada

Phone: (306) 966-5062; 5206; 966-2096
Facsimile: (306) 966-4796

APPROVAL PAGE

Revisions to an existing SOP, addition of an SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By:	Alan Blankenship, Markus Hecker and John P. Giesy	Date: _____
Supervisor Review By:	_____	Date: _____
Reviewed By: (QA Coordinator)	_____	Date: _____

DEFINITIONS AND ACRONYMS

CCV	Continuing Calibration Verification
COC	Chain of Custody
CQAP	Chemical Quality Assurance Plan
DOC	Dissolved Organic Carbon
EAL	Environmental Analytical Laboratory
EDD	Electronic Data Deliverable
GC/MS	Gas Chromatography/Mass Spectrometry
ICV	Initial Calibration Verification
IDs	Identifications
LCS	Laboratory Control Sample
LIMS	Laboratory Information Management System
QA	Quality Assurance
QAC	Quality Assurance Coordinator
QC	Quality Control
SOP	Standard Operating Procedure
SAP	Sampling Analysis Plan
TOC	Total Organic Carbon

TABLE OF CONTENTS

1.0 PURPOSE

The purpose for data package review is to ensure that final results reported are an accurate representation of the raw data generated during analysis. Data packages must function as stand-alone units. They must contain all information necessary to verify the reported results and to completely document the quality control procedures utilized during the analysis. Any deviations from the written protocol and/or quality control procedures which do not meet the documented limits must be clearly noted in the data package.

2.0 SCOPE AND APPLICATION

Data package review is applicable to all data packages generated by the Environmental Toxicology Laboratory at University of Saskatchewan (ETL) in conjunction with the Upper Columbia River White Sturgeon Work Plan. Two levels of review will be performed on each data package prior to submission of the data package to the Quality Assurance Coordinator. The first level of review will be performed by the primary analyst (analyst who performed the analysis or his/her designee). The second level of review is to be performed by the lead supervisor or their designee. Both levels of data package review must be documented utilizing the appropriate checklist. A Quality Control (QC) review will be performed at a frequency of 20% of samples.

3.0 SAFETY CONSIDERATIONS

All personnel shall adhere to prudent safety practices as specified in the project Health and Safety Plan (HASP).

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

The data package reviewers will require basic office equipment including, at a minimum, pens, a calculator and a computer. The computer must be loaded with Reflections for access to the LIMS software and should have the ability to review raw analytical data when required.

5.0 METHOD, PROCEDURES, AND REQUIREMENTS

Three levels of data review will routinely be performed:

Analyst Review

Technical Review

Quality Assurance Review

5.1 Primary Analyst Review

Once the data package has been generated, in accordance with ETL SOPs, the analyst, or their designee, will perform the first level of review. This review will verify the completeness of the data package prior to submission for technical review.

A. Review the Lot Folder Tracking Form. Verify that the header information is correct. Verify that the collection and analysis dates for samples are correct.

B. Each package will contain a data package checklist appropriate for the method performed. Verify that the data package includes all required forms as listed on the appropriate data package checklist. Verify that the data package is assembled in the order detailed on the appropriate data package checklist. Verify that the review of the contents have been performed by checking off each specific item on the appropriate data package checklist.

C. The primary analyst must verify each item on the appropriate Review Checklist. The review of each item must be noted by checking the appropriate item on the checklist.

D. Review the LIMS Worklist printout and the client and internal Chains-of-Custody (COCs). Verify that all samples on the client and internal COCs match the samples on the worklist and that there are no transcription errors or omissions.

E. Verify that there are no transcription errors on the LIMS worklist printout by checking the results against the raw data.

F. Check all QC sample results. Ensure that all quality control samples met acceptance criteria specified in the appropriate method SOP. If any QC samples do not meet criteria, verify that this is noted in the Case Narrative and also in the Analyst Comments section of the data package checklist. Verify that there is sufficient explanation regarding data acceptability**.**

G. Verify that all unused lines and entries on all forms have been lined out, dated, and initialed.

H. Verify that the correct calibration standards were used for quantitation.

I. Verify that all raw data for the analyses are included.

J. Verify correct calculation of results by recalculating the reported result on the Example Calculation Form.

K. Verify that all pages in the data package that require analyst signatures have been signed and dated by the analyst.

L. Document all variations from the SOP or problems with the analytical run in the Case Narrative.

M. If applicable, verify that the data package is consecutively paginated and that an EDD has been generated.

5.2 Technical Review

The technical review is the second level review and is performed by the Lead Supervisor or their designee. The technical review is performed to confirm the completeness of the package as submitted by the analyst and to verify the technical validity of the reported results.

A. Review the Lot Folder Tracking Form. Verify that all header information is included and is accurate. Review the items listed in the Case Narrative Form and add pertinent information, as appropriate. Sign and date the Lot Folder Tracking Form for Technical Review.

B. Each package will contain a data package checklist appropriate for the method performed. Verify that the data package includes all required forms as listed on the data package checklist. Verify that the data package is assembled in the order detailed on the appropriate data package checklist. Verify that the primary analyst has checked off all applicable items on the appropriate data package checklist.

C. Each data package will contain the appropriate Method Review Checklists. Verify that the primary analyst has completely and correctly filled out the Review Checklists. Verify that the primary analyst has signed and dated the checklist. Verify that items listed on the form have been included in the data package and that all information is accurate. Sign and date the Review Checklists as the Reviewer.

D. Review the LIMS worklist printout. Verify that there were no transcription errors for the reported results by reviewing the raw data. Verify that all QC sample recoveries are reported correctly. Sign and date the LIMS report as the Reviewer.

E. Review the Internal Chain-of-Custody to ensure that custody was maintained within the laboratory. Signatures and dates must be present for all exchanges of samples between personnel. Verify that all client COC are included for all samples contained on the Internal COC.

F. Review the Sample Preparation Form, if applicable. This form must include preparation information for all samples present in the analytical run(s). Verify that the following has been completed: all header information, sample IDs, sample initial and final weights or volumes, units, solvent used with manufacturer's name and lot number, spike volumes and solution IDs. Ensure that sample preparation steps and holding time requirements have been met. Verify that the spiking solutions are traceable to certified reference materials and the traceability has been clearly documented in the data package. Verify that none of the stock or working standards

used for spiking have expired. Verify that the source used for the LCS is different from the source used for the initial calibration standards. The source for the Matrix Spike (MS) may be from the same source as the LCS or the initial calibration standards. All unused lines must be crossed out, initialed, and dated.

G. Review the Additional Sample Preparation Information Form, if applicable. The steps involved in the preparation process must be clearly defined with all initial and final volumes clearly stated. All unused lines must be crossed out, dated and initialed.

H. Review the Sample Preparation Comments Form, if applicable. Verify that the header information is complete and accurate. Verify that the analyst included any comments regarding the sample preparation process which may affect the results and which deviate from the specified method. If there were no reportable instances which affect the data, verify that the analyst indicates this. All unused lines must be crossed out, dated and initialed by the analyst.

I. Review the Sample Extract Dilution Form, if applicable. Verify that the header information is complete and accurate. Verify that the Sample IDs and extract and solvent volumes and units are correct. Verify that the resulting dilution factors are correctly calculated. All unused lines must be crossed out, dated and initialed by the analyst.

J. Review the Solvent Purity Form, if applicable. Verify that the header information is complete and accurate. Verify that any solvents used for the method have had the solvent purity verified. As laboratory deionized water is continuously monitored, documentation using this form is not required.

K. Verify the calibration of the instrument. Documentation of the calibration may consist of the Calibration Form, instrument calibration reports, or the use of a spreadsheet or other documentation specified in the method SOP. Raw responses must be checked for accurate data transcription and acceptable calibration results. Verify that all calibration information presented on the raw data has been accurately transcribed onto the calibration forms. Verify that all instrument calibration criteria (initial calibration verification (ICV), continuing calibration verification (CCV), instrument drift checks, etc.) meet the requirements detailed in the LQCP and/or method SOP. For daily calibration checks, verify that the daily standard is checked against the correct initial calibration. Any manual integrations of calibration standards require clear identification, and an explanation for the use of manual integration.

L. Verify that all calibration and QC standard sources meet the requirements of the LQCP and associated method SOP. Verify that the calibration solutions are traceable to certified reference materials and the traceability has been clearly documented in the data package.

M. Verify that all associated raw data are included in the data package. Review the responses for all sample and QC analyses. Raw responses must be checked for accurate data transcription, if applicable. If a spreadsheet is used for interpretation of raw data, check for accurate data transcription. Confirm analysis holding time is within requirements. Verify that final concentrations have been calculated correctly by checking 10% of reported results. Check raw data for obvious problems, this may include elevated baselines, peak tailing, retention-time shifts, interfering ions, etc.

N. Verify that the analysis was in control by evaluating the recoveries observed for the QC samples. The method blank result should be below the method specific limits (MDL, PQL, MRL, etc). If there are concentrations present in the method blank at levels above the method specific limits, then professional judgement must be used to determine if the data are acceptable. This must be noted on the Case Narrative.

O. Verify that a matrix spike was performed and is included in the Data Package. Verify that the matrix spike recoveries are acceptable.

P. Verify that all standards used for the sample preparation and analysis have not expired. Verify that all required stock and working standards logbook pages are included. Verify that all standards can be traced back to a certified standard reference material and that all Certificates of Analysis are included.

Q. Verify that the report is consecutively paginated and that the EDD has been generated. If the report is not paginated and/or the EDD is not generated, perform these functions.

R. Although each data package is a stand-alone entity assessment of results particularly for laboratory control samples and certified reference materials should be compared to those of preceding data packages to detect possible trending of data with time. Should any trending be detected the data in all packages should be examined closely to determine the cause of any trends observed.

5.3 Quality Control (QC) Review

A. A QC review will be performed at a frequency of 20% of samples. The QC review will be performed utilizing the QC Lot Folder Review Checklist. For each portion of the data package to be reviewed, check each item listed on the QC Lot Folder Review Form to ensure completeness and accuracy.

B. If QC or technical discrepancies are identified in the 20% data package review, the QC reviewer should use their professional judgement in determining whether more than 20% of the data packages should be reviewed.

C. Verify that anomalies, variations, or problems are stated in the Case Narrative Section.

D. Verify that all necessary forms are included in the data package.

E. Verify that the field COC, Lab COC, LIMS worklist and Chain-of-Custody information is complete and accurate.

F. Verify that all sample preparation information is complete and accurate, and that sample prep and analytical holding time requirements have been met.

G. Verify that the calibration information is complete and accurate.

H. Verify that the sample response information is complete and accurate.

I. Verify that method blank, laboratory control spike, and matrix spike samples were performed and accurately reported. Verify that the QC samples meet the method specific criteria.

J. Sample integrity and traceability will be assessed in 2 samples per data package by performing a full audit trail analysis. The audit trail will track the sample documentation from field collection through final reporting and will include verification of sample documentation, sample container labeling and all COC and analytical procedures.

K. Sign and date the QC Review Checklist.

L. Upon completion of the QC review, any discrepancies in the data package should be brought to the attention of the Lead Supervisor for resolution.

6.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

A. The primary analyst shall document any anomalies and/or deviations from the specified method in the appropriate sections of the data package and list them in the Case Narrative Form. The primary analyst will sign and date any forms as the analyst.

B. The technical reviewer will record any problems noted during the technical review. The technical reviewer will return the data package to the analyst for corrections prior to submission of the data package. The technical reviewer must sign and date all forms as the reviewer.

C. The technical reviewer, or their designee, will paginate the report.

D. Generation of EDDs will be performed by the technical reviewer or by a designee of the technical reviewer or lead supervisor. As the nature of the EDD will vary considerably for each sample type and analytical procedure it is not possible to provide a definitive description of specific EDDs. EDDs will take the format of

summary tables which may be directly extracted from the data package but may also consist of scanned documents in electronic format suitable for electronic storage, transmission and retrieval. QC procedures for summary tables will be determined based on the method of generation. EDDs will be provided in a format that will allow them to be suitably protected from electronic manipulation of the data.

E. The QC reviewer will document any findings on the QC Lot Folder Review Checklist and notify the Lead Supervisor(s) and primary analyst.

7.0 RESPONSIBILITIES

Individuals and their project responsibilities are identified in a work plan or QAPP for each project. Any changes in personnel or their responsibilities will be noted in a protocol amendment and place on file with other project records.

8.0 REFERENCES

Field and Laboratory Policies and Procedures Manual, 1996, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824.

Data Quality Objectives Process for Superfund, Office of Emergency and Remedial Response, EPA 540-R-93-071, September 1993, United States Environmental Protection Agency, Washington DC 20460.

APPENDIX D

DERIVATION OF SURFACE WATER ANALYTICAL CONCENTRATION GOALS AND PROPOSED LABORATORY REPORTING AND DETECTION LIMITS BASED ON ECOLOGICAL SCREENING CRITERIA AND AVAILABLE DATA FOR THE SITE

Appendix D. Derivation of Surface Water Analytical Concentration Goals and Proposed Laboratory Reporting and Detection Limits Based on Ecological Screening Criteria and Available Data for the Site

		Ecological Screening Criteria							Proposed	
		Chronic EPA AWQC (µg/L)	Ecology Chronic WQS (µg/L)	Colville Confederated Tribes, Aquatic Life Chronic Criteria (µg/L)	Spokane Tribe of Indians, Aquatic Life Chronic Criteria (µg/L)	Paulson et al. (2006) (USGS) Reporting Limits (µg/L)	Risk-Based Concentration Values (Woodbury 2008, pers. comm.)	Analytical Concentration Goal (µg/L) [a]	MDL (µg/L)	MRL (µg/L)
Conventional Parameters	Alkalinity	NA	NA	NA	NA	NA	NA	NA	1000	2000
	DOC	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Hardness	NA	NA	NA	NA	NA	NA	NA	700	2000
	TDS	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TSS	NA	NA	NA	NA	NA	NA	NA	NA	NA
	TOC	NA	NA	NA	NA	NA	NA	NA	40	500
	pH	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Silicon dioxide (silica) (dissolved)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cations/Anions	Calcium	NA	NA	NA	NA	1,000	NA	1,000	30	50
	Chloride	230,000	230,000	230,000	230,000	NA	NA	46,000	9.0	200
	Fluoride	NA	NA	NA	NA	NA	52	**52**	6.0	200
	Magnesium	NA	NA	NA	NA	20	NA	20	0.70	20
	Potassium	NA	NA	NA	NA	500	NA	500	1.3	4
	Sodium	NA	NA	NA	NA	50	NA	**50**	50	100
	Sulfate	NA	NA	NA	NA	10,000	NA	10,000	7.0	200
Nutrients	Ammonia	NA	NA	NA	NA	NA	NA	NA	20	50
	Total Phosphorus	NA	NA	NA	NA	NA	NA	NA	2	10
	Nitrate	NA	NA	NA	NA	NA	NA	NA	3	100
	Nitrite	NA	NA	NA	NA	NA	NA	NA	3	100
Common Metals and Metalloids [b]	Aluminum	87	NA	87	87	15	23	23	0.30	2.0
	Antimony	NA	NA	NA	NA	0.30	0.34	0.34	0.030	0.050
	Arsenic	150	190	150	150	1.0	0.013	**0.013**	0.080	0.50
	Barium	NA	NA	NA	NA	1.0	3.3	3.3	0.020	0.050
	Beryllium	NA	NA	NA	NA	0.050	0.029	0.029	0.0080	0.020
	Cadmium	0.25	0.77 [c]	0.19 [c]	0.77 [c]	0.10	0.039	0.039	0.0080	0.020
	Chromium	74	128 [c]	53 [c]	**53**	5.0	100.0	10.6	0.070	0.20
	Cobalt	NA	NA	NA	NA	0.10	0.025	0.025	0.0050	0.020
	Copper	9.0	8.1 [c]	6.4 [c]	**6.4**	0.50	34	1.28	0.020	0.10
	Iron	1,000	NA	1,000	1,000	250	600	600	3	20
	Lead	2.5	1.6 [c]	1.6 [c]	**1.6**	0.25	15	0.32	0.0090	0.020
	Manganese	NA	NA	NA	NA	5.0	0.33	0.33	0.020	0.050
	Mercury	0.80	0.012	0.80	0.012	NA	0.000000089 [d]	**0.000000089** [d]	0.0001	0.00025
	Nickel	52	112 [c]	37 [c]	37 [c]	0.40	17	17	0.070	0.20
	Selenium	5.0	20	5.0	5.0	5.0	4.3	4.3	0.40	1.0

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	Silver	1.6 [c, e]	1.7 [c, e]	1.6	1.7 [c, e]	15	4.3	0.3	0.0090	0.020
	Thallium	NA	NA	NA	NA	0.20	0.06	0.06	0.0030	0.020
	Vanadium	NA	NA	NA	NA	2.5	0.86	0.86	0.080	0.20
	Zinc	120	74 [c]	84 [c]	74	2.5	260	15	0.10	0.50
Other Metals and Metalloids	Bismuth	NA	NA	NA	NA	0.20	NA	0.20	0.020	0.10
	Boron	NA	NA	NA	NA	NA	130	130	0.3	0.5
	Cerium	NA	NA	NA	NA	0.050	NA	0.050	0.020	NA
	Cesium	NA	NA	NA	NA	0.020	NA	0.020	0.020	NA
	Dysprosium	NA	NA	NA	NA	0.040	NA	0.040	0.020	NA
	Erbium	NA	NA	NA	NA	0.025	NA	0.025	0.020	NA
	Europium	NA	NA	NA	NA	0.025	NA	0.025	0.020	NA
	Gadolinium	NA	NA	NA	NA	0.025	NA	0.025	0.020	NA
	Gallium	NA	NA	NA	NA	0.050	NA	0.050	0.10	NA
	Germanium	NA	NA	NA	NA	0.250	NA	0.250	0.10	NA
	Gold	NA	NA	NA	NA	NA	NA	NA	0.020	NA
	Holmium	NA	NA	NA	NA	0.025	NA	0.025	0.020	NA
	Indium	NA	NA	NA	NA	NA	NA	NA	0.020	0.10
	Lanthanum	NA	NA	NA	NA	0.10	NA	0.10	0.020	NA
	Lithium	NA	NA	NA	NA	4.5	17	17.00	0.050	NA
	Lutetium	NA	NA	NA	NA	0.50	NA	0.50	0.020	NA
	Molybdenum	NA	NA	NA	NA	2.0	4.3	2.000	0.030	0.050
	Niobium	NA	NA	NA	NA	1.0	NA	1.0	0.020	NA
	Neodymium	NA	NA	NA	NA	0.1	NA	0.1	0.020	NA
	Praseodymium	NA	NA	NA	NA	0.1	NA	0.1	0.020	NA
	Rubidium	NA	NA	NA	NA	0.05	NA	**0.050**	0.10	NA
	Samarium	NA	NA	NA	NA	0.09	NA	0.090	0.02	NA
	Scandium	NA	NA	NA	NA	3.0	NA	3.0	0.10	NA
	Silicon (Silica)	NA	NA	NA	NA	1.0	NA	1.0	NA	NA
	Strontium	NA	NA	NA	NA	2.5	520	520	0.50	10
	Tantalum	NA	NA	NA	NA	0.10	NA	0.10	0.020	NA
	Tellurium	NA	NA	NA	NA	NA	NA	NA	0.100	NA
	Terbium	NA	NA	NA	NA	0.10	NA	0.10	NA	NA
	Thorium	NA	NA	NA	NA	1.0	NA	1.0	0.020	NA
	Thulium	NA	NA	NA	NA	0.045	NA	0.045	0.020	NA
	Tin	NA	NA	NA	NA	NA	NA	NA	0.040	0.1

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		Chronic EPA AWQC (µg/L)	Ecology Chronic WQS (µg/L)	Colville Confederated Tribes, Aquatic Life Chronic Criteria (µg/L)	Spokane Tribe of Indians, Aquatic Life Chronic Criteria (µg/L)	Paulson et al. (2006) (USGS) Reporting Limits (µg/L)	Risk-Based Concentration Values (Woodbury 2008, pers. comm.)	Analytical Concentration Goal (µg/L) [a]	MDL (µg/L)	MRL (µg/L)
	Titanium	NA	NA	NA	NA	2.5	NA	2.5	0.040	NA
	Tungsten	NA	NA	NA	NA	0.5	NA	0.5	0.020	NA
	Uranium	NA	NA	NA	NA	0.50	2	2.000	0.0050	0.020
	Ytterbium	NA	NA	NA	NA	0.025	NA	0.025	0.020	NA
	Yttrium	NA	NA	NA	NA	0.050	NA	0.050	0.020	NA
Stable Isotopes	Deuterium	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Oxygen-18	NA	NA	NA	NA	NA	NA	NA	NA	NA

Notes:

Analytical concentration goals (ACGs) for gallium and rubidium are slightly lower (0.5 times) than their associated anticipated MDLs, and may be achievable with minor method modifications.

Revisions to ACGs may be appropriate following input from EPA on human health risk assessment data needs.

AWQC = ambient water quality criterion

MDL = method detection limit

MRL = method reporting limit

NA = not available

WQS = water quality standards

[a] ACGs are one-fifth of lowest value of the screening benchmarks and historical reporting limits for the site, unless Woodbury (2008, pers. comm.) is the lowest value, in which case they are used in their entirety OR unless Paulson et al. (2006) was the only reference, in which case the Paulson et al. (2006) values were used in their entirety.

[b] Surface water samples will be analyzed for total and dissolved metals and metalloids.

[c] Criteria are hardness or pH dependent and are calculated using the means of those parameters from the Ecology (2006) surface water data. Mean hardness = 66.89 mg/L, Mean pH = 8.11 s.u., Mean temperature = 9.5°C.

[d] The risk-based concentration (RBC) for mercury in surface water is based on human consumption of fish (RBC_{FC}). Although the RBC_{FC} is not attainable, risks due to fish consumption will be addressed by directly analyzing fish samples. The direct use of the surface water data in the human health risk assessment will be to assess drinking water ingestion and the RBC for this pathway of 2.6×10^{-4} mg/L will be attained by the ACG. Low-level mercury methods are expected to provide MRLs below one-fifth of the lowest screening value (0.012 µg/L) for ecological risk assessment.

[e] Value represents the acute criterion; no chronic criterion exists for this analyte.

References

Ecology. 2006. Water quality standards for surface waters of the state of Washington, 28 Chapter 173-201A. Amended November 20, 2006. Publication No. 06-10-091.29, Washington State Department of Ecology, Olympia, WA.

Paulson, A.J., R.J. Wagner, R.F. Sanzolone, and S.E. Cox. 2006. Concentrations of elements in sediments and selective fractions of sediments, and in natural waters in contact with sediments from Lake Roosevelt, Washington, September 2004. Open-file report 2006-1350. U.S. Department of the Interior, U.S. Geological Survey, Reston, VA. [Reporting limits compiled from Tables 23-25.]

Woodbury, L. 2008. Personal communication (memorandum to M. Tonel, EPA Region 10, Seattle, WA, and Marc Stifelman, EPA Region 10, Seattle, WA, dated April 23, 2008, regarding human health risk-based concentrations for surface water, fish tissue and sediment in support of sampling and analysis plan development). Syracuse Research Corporation, Denver, CO.