

Upper Columbia River Remedial Investigation and Feasibility Study:

**Quality Assurance Project Plan (QAPP) for an evaluation of the acute or chronic toxicity
of individual chemicals of interest to white sturgeon (*Acipenser transmontanus*) and
rainbow trout (*Oncorhynchus mykiss*) in water-only exposures**

USGS-Columbia Study Code 10-20-07

-07a: Sturgeon acutes

-07b: Sturgeon chronics

-07c: Trout acutes

-07d: Trout chronics

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DISCLAIMER: PROVISIONAL STURGEON TOXICITY DATA FROM THE U OF S OR
FROM THE USGS-COLUMBIA IN TABLE 6 OF THIS QAPP ARE SUBJECT TO CHANGE;
HENCE, THESE DATA CANNOT BE CITED, QUOTED, OR DISTRIUBTED. THESE
STURGEON TOXICITY DATA ARE ONLY BEING USED FOR THE PURPOSE OF
ESTABLISHING METAL EXPOSURE CONCENTRATIONS IN THE ACUTE AND
CHRONIC STURGEON EXPOSURES TO BE CONDUCTED BY USGS-COLUMBIA IN
2010.

SECTION A.1 QUALITY ASSURANCE PROJECT PLAN APPROVAL PAGE

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45		
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124 **ATTACHMENT 1: USGS-COLUMBIA DATA REJECTION CRITERIA AND**
125 **CORRECTIVE ACTION PROCEDURES FOR ELEMENTAL ANALYSES**
126

127 **A.3 DISTRIBUTION LIST**
128

129 The USGS-Columbia Project Manager will be responsible for distribution of this QAPP. The
130 following individuals will receive copies of the approved QAPP and any subsequent revisions:
131

- 132 Chris Ingersoll, USGS-Columbia
- 133 Ed Little, USGS-Columbia
- 134 Paul Heine, USGS-Columbia
- 135 Ryan Warbritton, USGS-Columbia
- 136 Robin Calfee, USGS-Columbia
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- 139 Chris Mebane, USGS-Boise
- 140 Helen Bottcher, USEPA Region 10
- 141 Gina Grepo-Grove, USEPA Region 10
- 142 Bruce Duncan, USEPA Region 10
- 143 Marc Greenberg, USEPA Environmental Response Team
- 144 Dan Audet, National Park Service
- 145 Marko Adzic, Teck
- 146 Markus Hecker, University of Saskatchewan

147
148 **A.4 PROJECT/TASK ORGANIZATION**
149

150 Roles and responsibilities of individuals, including Quality Assurance authority, and interfaces
151 with USEPA are listed in Table 1.
152

153 **A.5 PROBLEM DEFINITION/BACKGROUND**
154

155 **Overview** - This Quality Assurance Project Plan outlines procedures for conducting water-only
156 acute copper, cadmium, and zinc toxicity tests and chronic copper, cadmium, zinc, and lead
157 toxicity tests with white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus*
158 *mykiss*) by the USGS in Columbia Missouri (USGS-Columbia) during the summer of 2010. The
159 2010 USGS-Columbia studies are designed to fulfill data needs outlined in the white sturgeon

160 level of effort document (USEPA 2010). Additionally, the studies will complement: (1) acute
161 and chronic water-only toxicity tests conducted with white sturgeon by Markus Hecker
162 (University of Saskatchewan [U of S] in 2008, unpublished data) and (2) acute water-only
163 toxicity tests conducted with white sturgeon by USGS-Columbia in 2007 to 2009 (Ed Little,
164 USGS-Columbia, unpublished data).

165
166 **Background** - White sturgeon in the trans-boundary reach of the Columbia River have
167 experienced poor recruitment. While adult sturgeon have reportedly engaged in spawning
168 activities and eggs and early life stages of white sturgeon have been observed in the river,
169 limited numbers of young-of-the-year have been found in habitats considered to be suitable for
170 this life stage. In addition, hatchery-reared juvenile white sturgeon released into the Columbia
171 River exhibit normal survival, growth, and body condition. While the underlying causes of poor
172 recruitment have not yet been determined, a number of factors that may be contributing to this
173 problem have been identified (UCWSRI 2002) and include:

- 174
- 175 • Habitat degradation;
 - 176 • Water quality impairment;
 - 177 • Genetic bottlenecks; and,
 - 178 • Predation by introduced species.
- 179

180 In addition to these factors, limited acute or chronic toxicity data suggest that early life stages of
181 white sturgeon may be more sensitive to certain contaminants of interest (COIs), particularly
182 copper, cadmium, or zinc, than are other species that have been used to derive ambient water
183 quality criteria. However, insufficient information is currently available to define toxicity
184 thresholds for these metals (USEPA 2010). Acute copper toxicity tests conducted in 2007 to
185 2009 by USGS-Columbia (Ed Little, unpublished data) indicate copper is highly toxic to 30-d-
186 old (27 to 38 dph, at the onset of exogenous feeding) white sturgeon with 96-hour LC50
187 (concentrations estimated to be lethal to 50% of the test organisms) ranging from about 3 to 7 µg
188 Cu/L at a water hardness of about 100 mg/L (as CaCO₃) and dissolved organic carbon of about
189 0.5 mg C/L. By comparison, white sturgeon that were about 160 dph were far more tolerant, with
190 LC50 values of about 245 µg Cu/L. Acute and chronic toxicity studies conducted in 2008 by the
191 U of S (Markus Hecker, unpublished data) with copper, cadmium, and zinc by the U of S
192 (Markus Hecker, unpublished data) indicate that early life stages of white sturgeon are
193 particularly sensitive to copper, and that these effects are dependent on the life stage (e.g.,
194 exogenous-feeding juveniles tend to be the most sensitive). However, there is uncertainty in the
195 chronic metal effect concentrations generated in the 2008 U of S study due to elevated control
196 mortality of white sturgeon at the onset of exogenous feeding in the chronic water toxicity tests.

197
198 USEPA Upper Columbia River (UCR) Technical Team identified four investigations that should
199 be conducted to evaluate the effects of COI exposures on white sturgeon in the UCR (USEPA
200 white sturgeon level of effort (LOE); USEPA 2010). The relative priority of each investigation
201 identified in USEPA white sturgeon LOE (USEPA 2010) was:

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- Investigation 1: Evaluation of the toxicity of individual COIs in water to sturgeon (i.e., water-only toxicity tests conducted in the laboratory)
- Investigation 2: Evaluation of the toxicity of COIs in surface water collected from the UCR to sturgeon (i.e., toxicity tests conducted in the field, a.k.a. the “streamside” tests)
- Investigation 3: Evaluation of the toxicity of COIs in sediment or slag to sturgeon (toxicity tests conducted in the laboratory)
- Investigation 4: Evaluation of the toxicity of COIs in the diet of sturgeon (i.e., modeling or dietary toxicity tests conducted in the laboratory)

Studies conducted by the U of S in 2008 (Markus Hecker, unpublished data) and by USGS-Columbia in 2007 to 2009 (Ed Little unpublished data) have provided some information to address some of the data needs associated with Investigation 1 in the USEPA white sturgeon LOE (USEPA 2010). However, additional studies are needed to provide the data required to evaluate the relative sensitivity of select life stages of white sturgeon to water-borne COIs in the UCR and to determine how sturgeon sensitivity to key COIs compares to the sensitivity of other, better studied fish (i.e., rainbow trout). USEPA (2010) states that Investigation 1 is needed to answer the following questions:

- What concentrations of cadmium, copper, and zinc are acutely toxic to early life stages of white sturgeon and rainbow trout in simulated UCR water (i.e., water that has the highest potential for toxicity of metals, within the range of conditions known to occur within the UCR; based on the results of 96-hour toxicity tests; Endpoint: survival, loss of equilibrium)?
- What concentrations of cadmium, copper, lead, and zinc are chronically toxic to early life stages of white sturgeon and rainbow trout in simulated UCR water (i.e., water that has the highest potential for toxicity of metals, within the range of conditions known to occur within the UCR; based on the results of 28-day toxicity tests; Endpoints: survival, growth, biomass)?
- What are acute-to-chronic ratios of copper, cadmium, and zinc for white sturgeon and rainbow trout?

Information is needed by resource managers of the UCR on potential effects of COIs, including metals on the Lake Roosevelt ecosystem specifically in reference to white sturgeon. White sturgeon are threatened by failure of natural recruitment resulting in a population consisting primarily of aging fish which are gradually declining as fish die and are not replaced.

Toxicity tests conducted by USGS-Columbia in 2010 will further address data needs outlined in Investigation 1 of the USEPA white sturgeon LOE (USEPA 2010). Specifically, the objective of the 2010 USGS-Columbia studies will be to: (1) conduct acute (96-hour) water-only exposures to individual metals (copper, cadmium, and zinc) with white sturgeon and rainbow trout to

244 identify early life stages that may be particularly vulnerable to such exposures and the associated
245 effect concentrations; and, (2) conduct chronic water-only exposures to individual metals
246 (copper, cadmium, lead and zinc) with white sturgeon and with rainbow trout to identify effect
247 concentrations of these metals. Studies conducted by USGS-Columbia in 2010 will provide
248 additional acute and chronic toxicity data to better understand the sensitivity of white sturgeon to
249 exposures to individual metals. Potential effects of COI mixtures on sturgeon are being
250 evaluated: (1) in streamside toxicity tests (Investigation 2; U of S 2008 and 2009 studies) and (2)
251 in sediment and slag toxicity tests (Investigation 3; U of S study planned for 2010). Depending
252 on the outcome of the studies conducted by USGS-Columbia and by U of S, additional
253 laboratory toxicity tests may be needed to further evaluate the influence of mixtures on the
254 sensitivity of sturgeon to COIs in water or in sediment (USEPA 2010).

255

256 The USGS-Columbia studies will be conducted under the following five tasks:

257

- 258 Task 1: Obtain white sturgeon and rainbow trout eggs for toxicity testing;
- 259 Task 2: Conduct acute toxicity tests with white sturgeon and rainbow trout with copper,
260 cadmium and zinc;
- 261 Task 3: Conduct chronic toxicity tests with white sturgeon and rainbow trout with copper,
262 cadmium, zinc and lead;
- 263 Task 4: Evaluate the relative sensitivity of white sturgeon and rainbow trout to acute and
264 chronic exposures to individual metals; and,
- 265 Task 5: Evaluate the relative sensitivity of early life stages of white sturgeon to USEPA
266 ambient water quality criteria for copper, zinc, cadmium, or lead in the Upper
267 Columbia River.

268

269 Table 1 provides a summary of roles and responsibilities of key individuals associated with the
270 project. Table 2 provides a summary of conditions that will be used to culture and handle white
271 sturgeon and rainbow trout at the USGS-Columbia laboratory. Table 3 provides a summary of
272 the number of acute toxicity tests to be conducted in 2010 with white sturgeon and rainbow
273 trout. Table 4 provides a summary of test conditions that will be used to conduct the acute and
274 chronic toxicity tests with white sturgeon and rainbow trout (adapted from ASTM 2009a,b,c).
275 Table 5 provides a listing of daily activities for conducting the toxicity tests. Table 6 provides a
276 summary of historic acute and chronic toxicity data for white sturgeon and for rainbow trout.
277 Tables 7, 8 and 9 provide a summary a summary of measures of water quality and metal
278 chemistry for water samples collected from the toxicity tests. Table 10 provides a summary of
279 test acceptability requirements for the acute and chronic toxicity tests. **DISCLAIMER:**
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281 **COLUMBIA IN TABLE 6 OF THIS QAPP ARE SUBJECT TO CHANGE; HENCE, THESE**
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283 **TOXICITY DATA ARE ONLY BEING USED FOR THE PURPOSE OF ESTABLISHING**
284 **METAL EXPOSURE CONCENTRATIONS IN THE ACUTE AND CHRONIC STURGEON**
285 **EXPOSURES TO BE CONDUCTED BY USGS-COLUMBIA IN 2010.**

286

287 It is anticipated that the toxicity testing will be started by USGS-Columbia in July 2010 and will
288 continue through September 2010. USGS-Columbia will provide quarterly progress memos to
289 USEPA, the Participating Parties, and Teck American Incorporated (Teck). USGS-Columbia
290 will also provide the USEPA Project Manager with periodic written or verbal progress
291 summaries (e.g., by email or phone calls or by conference calls organized by USEPA).

292

293 USGS-Columbia will develop a final report summarizing the results of the 2010 studies. The
294 final report will include: (1) a summary of the data generated from the study (Tasks 1, 2, and 3)
295 and (2) interpretations of relationships between chemistry and toxicity (Tasks 4 and 5). It is
296 anticipated that an initial draft of the final report will be provided to USEPA, the Participating
297 Parties, and Teck by about January 2011 (timing of the completion of the initial draft of the final
298 report will depend on the timing and extent of comments received from USEPA on the summary
299 of the preliminary data generated for the project; see below).

300

301 Once USGS-Columbia receives comments on the initial draft of the final report from USEPA, or
302 other organizations that were provided with a courtesy copy of the initial draft of the final report,
303 USGS-Columbia will then conduct a peer review of the revised draft final report in accordance
304 with USGS publication requirements. The USGS peer review of the draft final report and the
305 associated data will be coordinated with review requirements identified by USEPA, the
306 Participating Parties, and Teck.

307

308 In advance of completing the USGS-Columbia final report, full data packages of draft data will
309 be provided by USGS-Columbia to USEPA (in the form of a memo) for all chemical and
310 biological measurements. These draft data packages will include sufficient information to
311 support full data validation, including summarized data with applied qualifiers, results for QC
312 samples, initial and continuing calibration data, instrument printouts and run logs, sample
313 preparation logs, and copies of datasheets and associated study folders (see Section A.10). CH₂M
314 Hill on behalf of USEPA will develop a data validation report based on the information provided
315 in these data packets. The USGS-Columbia will then use validated data as summarized in the
316 USEPA data validation report in the preparation of the USGS-Columbia final report.

317

318 USGS-Columbia draft chemistry and biological data will be provided to USEPA with 90
319 calendar days of completion of the final analyses in order to initiate development of a data
320 validation report by CH₂M Hill (i.e., after all final data collection activity for chemical and
321 biological data). It is anticipated that the data validation report will be completed under the
322 direction of USEPA by about 60 days after receiving chemistry and biological data from USGS-
323 Columbia

324

325 Personnel from the U of S and from USGS-Columbia will routinely exchange information on the
326 status of the ongoing sturgeon-trout water toxicity studies conducted at the USGS-Columbia and
327 the ongoing sturgeon sediment toxicity studies conducted at the U of S in 2010 (Hecker 2010). It

328 is anticipated that these exchanges will include frequent phone calls, emails, preliminary data
329 summaries, and potential site visits.

330

331 **A.6 TASK DESCRIPTIONS**

332

333 **Task 1: Obtain white sturgeon and rainbow trout eggs for toxicity testing**

334

335 Conditions for culturing and handling of white sturgeon and rainbow trout are summarized in
336 Table 2. White sturgeon will be obtained from the State of Washington Columbia Basin
337 Hatchery within about 3 days of fertilization. Rainbow trout will be obtained from Ennis
338 National Fish Hatchery, Ennis, Montana. White sturgeon or rainbow trout will be obtained by
339 spawning at least 2 females and 2 males. Culturing and testing of fish will comply with all
340 applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and will
341 comply with USGS-Columbia guidelines for the humane treatment of test organisms during
342 culture and testing. Whenever possible, procedures have been designed to avoid or minimize
343 discomfort, distress or pain to animals. By design, however, fish will be subject to conditions
344 that might result in pain, distress, or death.

345

346 **Task 2: Conduct acute toxicity tests with white sturgeon and rainbow trout with copper,** 347 **cadmium, and zinc**

348

349 A series of consecutive 96-hour acute toxicity tests will be conducted with early life stages of
350 white sturgeon and rainbow trout starting with about 1 dph fish to about 90 dph fish (Table 3).
351 The toxicity tests will be conducted in accordance with guidance provided in ASTM (2009a).
352 Test conditions for conducting the acute toxicity tests are summarized in Table 4. Table 5
353 provides a listing of daily activities for conducting the acute toxicity tests. Tables 7, 8 and 9
354 provide a summary of measures of water quality and metal chemistry for water samples collected
355 from the acute toxicity tests. Table 10 provides a summary of test acceptability requirements for
356 the acute toxicity tests.

357

358 Metals to be evaluated in the acute toxicity tests will be copper, cadmium, and zinc. While lead
359 has been identified as a COI in the UCR, no acute testing will be conducted with lead due to the
360 low water solubility of lead in waters representative of the UCR (USEPA 2010). The acute
361 toxicity tests will be conducted in intermittent proportional diluters with toxicant stock solutions
362 delivered with each cycle of the diluters by Hamilton syringe pumps (Hamilton, Reno, NV,
363 USA; Wang et al. 2007; Table 4). The exposure water will be water adjusted to be representative
364 of water quality conditions of the UCR (e.g., water hardness 100 mg/L as CaCO₃, dissolved
365 organic carbon of 0.5 mg C/L; Table 4). Concentrations of copper, cadmium, or zinc will be
366 adjusted at each interval of the exposures to bracket estimated effect and no effect concentrations
367 for each metal through the early development of white sturgeon and rainbow trout.

368

369 Life stages of white sturgeon and rainbow trout to be tested in the acute exposures are
370 summarized in Table 3. Some of these life stages have been previously tested by USGS-
371 Columbia (Ed Little, unpublished data) and by the U of S (Markus Hecker, unpublished data).
372 Endpoints to be measured daily in the acute exposures will include survival and changes in
373 behavior (Table 4). Behavior will include endpoints such as: loss of equilibrium or change in fish
374 location in aquaria, feeding, activity, coloration, or respiration (see Figure 1 in ASTM 2009c;
375 e.g., photo documentation in the form of a video may also be used to qualitatively document
376 potential behavioral responses).

377
378 It is anticipated that the toxicity testing of white sturgeon and rainbow trout will be conducted
379 sequentially at USGS-Columbia (i.e., sturgeon testing starting in about July 2010 and trout
380 testing started in the fall of 2010). The benefit of conducted the toxicity tests sequentially will be
381 that the concentrations of metals can be adjusted to bracket the exposure concentrations for each
382 species (Table 6).

383
384 **Task 3: Conduct chronic toxicity tests with white sturgeon and rainbow trout with copper,**
385 **cadmium, zinc, and lead**

386
387 White sturgeon frequently exhibit high rates of mortality during the onset of exogenous feeding
388 that can invalidate standard test criteria for survival, and obscure the dose response to exposure.
389 In an effort to control for this early mortality, a two-phase (biphasic) study will be conducted in
390 four diluters with copper, cadmium, zinc, and lead. Stage 1 Exposures will be started with fish at
391 about 1 dph and continue through about 21 days and Stage 2 Exposures will be started with
392 naïve fish about 28 dph and continue for 28 days (Table 4). To assist in the interpretation of the
393 chronic toxicity data generated by U of S in 2008, Continuous 56-day Toxicity Tests with
394 copper, cadmium, zinc, and lead will be conducted in these same four diluters starting with about
395 1 dph fish continuing past the onset of exogenous feeding (i.e., 4 replicate chambers for the
396 Stage 1 and Stage 2 Biphasic Exposures and 4 replicate chambers for the Continuous Chronic
397 56-day Test). It is anticipated that control survival of white sturgeon in this Continuous Chronic
398 56-day Test may be low due to elevated mortality at the onset of exogenous feeding. The goal of
399 the Continuous Chronic 56-day Tests will be to compare 56-day effect concentrations for white
400 sturgeon or rainbow trout to effect concentrations in the: (1) USGS-Columbia Stage 1 21-day
401 Biphasic Exposures; (2) USGS-Columbia Stage 2 28-day Biphasic Exposures; and, (3) U of S
402 Continuous Chronic 66-day Tests started with freshly fertilized eggs. Test conditions for
403 conducting the chronic toxicity tests are summarized in Table 4. Table 5 provides a listing of
404 daily activities for conducting the chronic toxicity tests. Tables 7, 8 and 9 provide a summary of
405 measures of water quality and metal chemistry for water samples collected from the chronic
406 toxicity tests. Table 10 provides a summary of test acceptability requirements for the chronic
407 toxicity tests.

408
409 The chronic toxicity tests will be conducted in intermittent proportional diluters with toxicant
410 stock solutions delivered with each cycle of the diluters by Hamilton syringe pumps (Hamilton,

411 Reno, NV, USA; Wang et al. 2007; Table 4). The exposure water will be water adjusted to be
412 representative of water quality conditions of the UCR (e.g., water hardness 100 mg/L as CaCO₃,
413 dissolved organic carbon of 0.5 mg C/L; Table 4). Concentrations of copper, cadmium, zinc, or
414 lead to be tested will be based on effect concentrations anticipated to bracket the sensitivity of
415 white sturgeon or rainbow trout (Table 6).

416
417 Chronic tests started with about 1 dph fish through the swim-up juvenile life stage will have five
418 medium-size pieces of gravel (1- to 2-cm diameter) placed in each chamber (to reduce activity of
419 fish through the hiding stage; Ed Little, unpublished data). Endpoints to be measured daily in the
420 chronic exposures will include survival and changes in behavior (Table 4). Behavior endpoints
421 will include measurements such as: loss of equilibrium or change in fish location in aquaria,
422 feeding, activity, coloration, or respiration (see Figure 1 in ASTM 2009c). Endpoints to be
423 measured at the end of each biphasic 28-day exposure and at the end of each chronic continuous
424 test) will include: (1) growth (average total length, average wet weight, average dry weight);
425 and, (2) biomass (total wet or dry weight of surviving fish in each replicate). Wet weight of fish
426 by replicate will be determined by gently blotting fish on a dry paper towel before weighing the
427 fish. Fish will be preserved in 10% formalin for morphological measurements (e.g., external
428 gross pathology using methods developed by the U of S; Markus Hecker, personal
429 communication) and for measurement of length of individual fish. Dry weigh of fish by replicate
430 will be determined after morphological and length measurements have been completed by drying
431 surviving fish to a constant weight at 60°C (e.g., for 24 h of drying).

432
433 The Stage 1 Exposures in the first half of the Chronic Biphasic Tests started with about 1 dph
434 fish will be conducted for about 21 days (e.g., until about 50% of the juveniles begin to swim
435 up). Exposures in the first half of the Chronic Biphasic Test started with about 1 dph fish may be
436 continued for more than 21 days if control survival has not dropped below about 80%, but would
437 be discontinued before the start of the Stage 2 of the 28-day Biphasic Tests started with
438 exogenous-feeding juveniles (about 28 dph; Table 5).

439
440 **Task 4: Evaluate the relative sensitivity of white sturgeon and rainbow trout to acute and**
441 **chronic exposures to individual metals**

442
443 The results of the toxicity tests will be used to evaluate the relative sensitivity of white sturgeon
444 (i.e., compared to rainbow trout) to the selected COIs (cadmium, copper, zinc, or lead) evaluated
445 in this study. These comparisons will be based on measures of survival, growth of surviving fish
446 (length, weight), or biomass of surviving fish (total weight of surviving fish in each replicate).
447 Statistical comparisons will be made using no-observable-effect concentration (NOEC) or
448 lowest-observable-effect concentration (LOEC), chronic value (geometric mean of NOEC and
449 LOEC), and various regression procedures to calculate EC_p or LC_p (concentrations estimated to
450 cause a lethal or sublethal effect on a percentage of the population; ASTM 2009a,b,c; Toxicity
451 Relationship Analysis Program (TRAP) obtained from Russell Erickson, USEPA, Duluth MN).
452 Chemical characterization of the water samples will include measures of major cations, major

453 anions, dissolved organic carbon, and pH (for use in biotic ligand models (BLM) of toxicity and
454 chemistry data; e.g., USEPA 2007). Biotic ligand models for copper have been developed to
455 enable mechanistic modeling of copper bioavailability and acute toxicity as a function of water
456 quality variables (e.g., major cations, major anions, pH, dissolved organic carbon; USEPA
457 2007). Similarly, BLMs for zinc, cadmium, and lead (Robert Santore, Hydroqual Environmental
458 Engineers and Scientists, East Syracuse, NY; unpublished) will be used to evaluate relationships
459 between bioavailability and toxicity as a function of water quality variables in the acute and
460 chronic exposures.

461
462 Data generated from these studies will be used to calculate acute to chronic ratios and to evaluate
463 the applicability of water quality criteria, including criteria based on biotic ligand model
464 estimates to be protective of early life stages of white sturgeon.

465
466 **Task 5: Evaluate the relative sensitivity of early life stages of white sturgeon to USEPA**
467 **ambient water quality criteria for copper, zinc, cadmium, or lead in the Upper Columbia**
468 **River**

469
470 The metal effect concentrations for white sturgeon and rainbow trout will be evaluated in the
471 context of existing water quality criteria. More specifically, the level of protection offered by
472 the water quality criteria to these species for the chemicals tested will be evaluated following
473 procedures outlined in Wang et al. (2007, 2010).

474
475 **A.7 QUALITY OBJECTIVES, INDICATORS, AND CRITERIA FOR**
476 **MEASUREMENT DATA**

477
478 The parameters used to define data quality are accuracy, precision, completeness, comparability,
479 representativeness, and method sensitivity. Data quality indicators (DQIs; i.e., performance
480 criteria for measurement data) for accuracy, precision, comparability, and completeness are
481 listed in Table 8. The data quality objective will be to conduct the toxicity tests to conform to
482 ASTM standards for conducting acute or chronic toxicity test with fish (ASTM 2009a,b,c).

483
484 The precision and accuracy indicators specified in Table 8 are based on standard method
485 performance information, when available, and historical laboratory performance. Tables 8 and 9
486 contain a list of the parameters to be analyzed along with their applicable chemical analytical
487 methods and associated target method detection limit for metal analyses.

488
489 **A.7.1 Accuracy**

490
491 Accuracy is a measure of the bias of a system or measurement. It is the closeness of agreement
492 between an observed value and an accepted value. For this project, accuracy of chemical analysis
493 will be determined through the analysis of certified reference solutions (i.e., National Institute of
494 Standards and Technology (NIST) number) and spiked samples. Accuracy will be expressed as

495 percent recovery (ratio of measured concentration to certified concentrations, expressed as
496 percent). Method blanks will be used to measure contamination associated with sampling,
497 laboratory processing, and analyses. Acceptable accuracy for routine water quality analyses are
498 assured by (1) the calibration of the instruments used and (2) establishment of acceptable ranges.
499

500 For toxicity tests, no true accuracy measurements are possible because responses to toxicants
501 vary among tests due to many factors, including factors such as species, life stage, water quality,
502 and test conditions. Test acceptability will be determined using performance-based criteria
503 outlined by ASTM (2009a,b,c). Performance of organisms in negative controls (test water
504 without added toxicant) is commonly used as a performance indicator, with a minimum survival
505 of 90% recommended by ASTM (2009a) for acute toxicity tests with fish (Table 10). No
506 guidance is provide for survival of sturgeon in biphasic exposures (ASTM 2009b), but it is
507 anticipated that survival of sturgeon or trout in the controls will be at least 80% at the end of the
508 Chronic Biphasic Exposures (at the end of the Stage 1 21-d Exposures and at the end of the
509 Phase 2 28-day Exposures and at least 64% by the end of the Continuous Chronic 56-day Test
510 (Table 10). These performance-based standards will be applied directly to evaluate test
511 acceptability for white sturgeon water-only exposures.

512 513 **A.7.2 Precision** 514

515 Precision is a measure of mutual agreement among individual measurements of the same
516 property, usually under prescribed similar conditions. For this project, measures of analytical
517 precision will be determined by the analysis of laboratory duplicates. Laboratory replicates will
518 be prepared by splitting a sample in the laboratory, and carrying the subsamples through the
519 entire analytical process. Precision will be expressed in terms of the relative percent difference
520 (RPD). For all analysis where duplicates are performed, RPD will be calculated as follows:
521

$$522 \quad \text{RPD} = \frac{|C_1 - C_2|}{(C_1 + C_2)/2} \times 100$$

523 $C_1 =$ larger measured value
524 $C_2 =$ smaller measured value
525

526 **A.7.3 Completeness** 527

528 Completeness is a measure of the amount of valid data obtained from a measurement system
529 compared to the amount that was expected to be obtained under normal conditions. Target
530 completeness values are 90% for chemical analyses of water and 100% for toxicity tests
531 ancillary measurements. Completeness (C) is defined as follows for all measurements:

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$$\% C = 100 \times \frac{V}{n}$$

% C = percent completeness;
V = number of measurements judged valid; and,
n = total number of measurements attempted.

A.7.4 Representativeness

Representativeness expresses the degree to which data accurately and precisely represents a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition. Representativeness will be addressed primarily in the experimental design and through the selection of appropriate procedures. Representativeness also will be ensured by the proper handling and storage of samples and analysis within the accepted holding times so that the material analyzed reflects the material collected as accurately as possible (Table 9). Representativeness of data will be discussed, when appropriate, in deliverable reports.

A.7.5 Comparability

Comparability expresses the confidence with which one data set can be compared to another. For this project, comparability of the toxicity data generated at the USGS-Columbia and U of S laboratories will be evaluated by conducting a series of reference toxicant tests with white sturgeon under similar water quality conditions. In addition, comparability of water chemistry data will be assured through the use of consistent laboratory methods (Tables 8 and 9). Comparability of other data will be discussed, when appropriate, in the final report.

A.7.6 Sensitivity

Sensitivity is the capability of methodology or instrumentation to discriminate among measurement responses for quantitative differences of a parameter of interest. Sensitivity for chemical analyses will be described by the method detection limit, minimum concentration of a substance that can be measured and reported. Target detection limits for the parameters of interest are presented in Table 8 and are based on the applicable methods. Acute reference toxicant tests with copper will also be conducted periodically with subsets of organisms used for conducting acute or chronic toxicity tests with chemicals of interest (ASTM 2009a,b,c).

A.8 PROJECT NARRATIVE

See Section A.5.

574 **A.9 SPECIAL TRAINING REQUIREMENTS/CERTIFICATION**

575

576 No additional special training requirements are necessary for USGS-Columbia personnel to
577 conduct the toxicity tests outlined in Table 1 and the chemical analyses listed in Table 8.

578

579 **A.10 DOCUMENTATION AND RECORDS**

580

581 At the conclusion of all toxicity testing and chemistry analyses, USGS-Columbia will prepare a
582 final report to the USEPA. At a minimum, the final report will include the following
583 information:

584

- 585 • Summarized data for all toxicity tests (survival, growth, behavior, morphology), water
586 quality measurements, and chemical analyses;
- 587 • Description and results of QC checks;
- 588 • Description of analytical methods;
- 589 • Summaries of the biological observations;
- 590 • Description of procedures used to culture test organisms;
- 591 • Summary of any problems encountered and corrective actions;
- 592 • Description of any deviations from prescribed laboratory protocols;
- 593 • Description of changes to prescribed laboratory protocols; and,
- 594 • Data quality summary based on the results of USEPA data validation report, including
595 explanations of any qualifiers applied to the data as well as data usability implications for
596 qualified data (Section D.3).

597

598 All project supporting records and documents will be archived with USGS-Columbia after
599 completion of the final report.

600

601 In advance of completing the USGS-Columbia final report, full data packages of draft data will
602 be provided by USGS-Columbia to USEPA for all chemical and biological measurements. These
603 draft data packages will include sufficient information to support full data validation, including
604 summarized data with applied qualifiers, results for QC samples, initial and continuing
605 calibration data, instrument printouts and run logs, sample preparation logs, and copies of
606 datasheets and associated study folders. CH₂M Hill on behalf of USEPA will develop a data
607 validation report based on these data packages. The USGS-Columbia will then use validated data
608 as summarized in the USEPA data validation report in the preparation of the USGS-Columbia
609 final report.

610

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

613

- 614 • Exposure system maintenance logs and sample collection records including notebooks,
615 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

- 616 required to operate the exposure systems or to collect samples will be described in detail.
- 617 • Chain of custody records.
 - 618 • Corrective action reports.
 - 619 • Sample identification, treatment, matrix, and dilution factor (whichever is applicable).
 - 620 • Sample receipt and analysis dates.
 - 621 • Result/assessment and reanalyses (if necessary).
 - 622 • Final analyte concentration including reporting limit, laboratory qualifiers, and
 - 623 reanalyses.
 - 624 • Percent recovery of each compound in the matrix spike (MS) samples.
 - 625 • Relative percent difference (RPD) for all matrix spike/matrix spike duplicates (MSD).
 - 626 • MS/MSD and/or laboratory control sample (LCS)/LCS duplicate (LCSD) results.
 - 627 • Laboratory control sample results when analyzed.
 - 628 • Blank results for method blanks, experimental blanks, and equipment blanks.
 - 629 • Method blank summary indicating associated samples.
 - 630 • Case narrative.

631
632 For data validation, the following additional data will be required:

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

637
638 All data and logs will include the following information:

- 639 • Analyst's initials and date.
- 640 • Initial and final sample and extract volumes or weights and/or dilutions
- 641 • Condition of instrument (daily performance check).
- 642 • Documentation linking sample analysis to instrument calibration (where appropriate).
- 643 • Analysis start time of all experimental and quality control samples.
- 644 • Instrument run log showing analytical sequence.
- 645 • Dilutions performed and amount of sample analyzed.
- 646 • Experimental samples, quality control samples, and blanks clearly labeled.
- 647 • Quantification reports.
- 648 • Sample preservation (where applicable).

649
650 Paper copies of all these records will be retained. In addition, the laboratory will provide (1) an
651 electronic deliverable in Microsoft Excel format for all test results, and (2) an electronic backup
652 for all onsite and laboratory data generated.

653
654

655 **SECTION B: MEASUREMENT/DATA ACQUISITION**

656

657 **B.1 SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)**

658

659 See Table 4 and 7. Methods for data analysis will conform with procedures outlined in ASTM
660 (2009a,b,c).

661

662 **B.2 SAMPLING METHODS REQUIREMENTS**

663

664 Not applicable.

665

666 **B.3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS**

667

668 Applicable chain of custody forms will be used to transfer samples between USGS-Columbia
669 and other laboratories.

670

671 **B.4 ANALYTICAL METHODS REQUIREMENTS**

672

673 **B.4.1 Chemical Analyses of Water**

674

675 Concentrations of dissolved copper, cadmium, zinc, or lead in test water will be measured by
676 analysis of filtered samples: 20 ml of test water is drawn into a pre-cleaned polypropylene
677 syringe and dispensed through a filter disc (0.45- μ m pore diameter; USGS-Columbia SOP
678 P.566; Tables 8 and 9). Samples for copper, cadmium, zinc, and lead analysis will be acidified to
679 1% (v/v) with nitric acid and stored in pre-cleaned polyethylene bottles. Metal concentrations
680 will be determined by inductively-coupled plasma mass spectrometry (ICP-MS, Brumbaugh et
681 al. 2007).

682

683 **B.4.2 Water Quality Analyses**

684

685 Hardness, alkalinity, dissolved oxygen, ammonia, conductivity, and pH will be routinely
686 measured in water samples using methods described in Kemble *et al.* (1994) and APHA (2005;
687 Tables 8 and 9). Dissolved oxygen will be measured with an YSI Model 54A oxygen meter and
688 an YSI 5739 probe. Conductivity will be measured with an Orion 140 S-C-T Meter with an
689 014010 conductivity probe. Alkalinity and pH will be measured with an Orion EA940
690 Expandable Ionalyzer, Orion 917001 ATC probe, and Orion 8165BN combination pH probe.
691 Total hardness will be measured by EDTA titration. Total ammonia will be measured with an
692 Orion EA940 Expandable Ionalyzer and Orion 95-12 ammonia electrode. Dissolved organic
693 carbon, particulate organic carbon, major cations and major anions in test waters will be
694 determined using methods outlined in Tables 8 and 9.

695

696 **B.5 QUALITY CONTROL REQUIREMENTS**

697
698 Key quality control (QC) requirements are discussed in Section A.7 and listed in Tables 8 and 9.
699 See Attachment 1 for a description of the USGS-Columbia procedure for data rejection criteria
700 and corrective action procedures for elemental analyses (i.e., stocks and standards, calibration of
701 equipment, blanks, limits of detection, spikes, method precision, reference materials, duplicates,
702 background, procedural deviations, data review).

703 **B.6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE** 704 **REQUIREMENTS**

705
706
707 Instruments used to measure routine water quality characteristics during toxicity testing will be
708 maintained to ensure that they are in proper working order during the conduct of this study.
709 Preventative maintenance requirements described by the manufacturer in the instrumentation
710 manuals will be followed.

711 **B.7 INSTRUMENT CALIBRATION AND FREQUENCY**

712
713
714 All instruments used on this project will be traceable to the data collected and will be calibrated
715 before use. Information on calibration of instruments will be maintained at USGS-Columbia. As
716 a minimum, calibrations will include:

- 717
- 718 • Standards that are traceable to nationally recognized standard organization(s);
- 719 • Standards that are within their expiration date; and,
- 720 • Using standard concentrations that bracket the expected concentration of the sample(s).
- 721

722 **B.8 INSPECTION/ACCEPTANCE REQUIREMENTS FOR SUPPLIES AND** 723 **CONSUMABLES**

724
725 All certifications for standards used to calibrate instruments and all certifications for sample
726 container cleanliness will be maintained as part of the project record. Upon receipt of the
727 certifications, an inspection will be performed to accept the certifications. At a minimum, this
728 inspection will include:

- 729
- 730 • Verification that the certificate matches the standard or sample container; and,
- 731 • Signature on the certificate indicating acceptance.
- 732

733 **B.9 DATA ACQUISITION REQUIREMENTS (Non-Direct Measurements)**

734
735 Published and unpublished data used to establish chronic exposure concentrations for sturgeon
736 and trout are summarized in Table 6. All of the data summarized in Table 6 will be reviewed to
737 determine that these data meet the same measurement quality objectives as described in this
738 QAPP.

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B.10 DATA MANAGEMENT

Data recording practices in the laboratory will follow standard procedures of documentation. Data will be recorded in ink and will be signed and dated by the person making the entry. Changes to data will be initialed and dated by the person responsible for the change. All project supporting records and documents will be archived at USGS-Columbia after completion of the final report. See Section A.5 for a description of the data validation report to be prepared by USEPA. See Section D.2 for a description of electronic records and validation and verification of data entries in spreadsheets.

SECTION C: ASSESSMENT/OVERSIGHT

C.1 ASSESSMENTS AND RESPONSE ACTIONS

Assessments that will be performed for this project include:

- Verification of proficiency training of technicians participating on project. This will be performed by the USGS-Columbia Principal Investigators and will be conducted before starting testing;
- Data quality audit to verify QA/QC requirements were met. This review will be conducted on the preliminary data by the USGS and USEPA Project QA Officers and will be conducted before submission of final report; and,
- Technical review of data. This will be conducted by USGS-Columbia Principal Investigators and the USGS-Columbia Project Managers. This review will ensure that all laboratory records related to the test are completed and reviewed for completeness and accuracy.

All non-conforming conditions will be documented and corrective action will be documented and completed as necessary to ensure that data quality issues are minimized. All non-conforming conditions will be provided to the USEPA Project Manager (Table 1).

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. Corrective action procedures include prompt notification of the project contact (quality assurance manager) for any significant problems or discrepancies. The USGS-Columbia Project Managers are responsible for reporting any significant problems or discrepancies that occur as analyses are conducted to the USEPA Project Manager. The USGS-Columbia Project Managers are also responsible for ensuring that corrective action is taken, where appropriate, to prevent the reoccurrence of similar problems or discrepancies. In addition, the final report will include a case narrative that

781 discusses any significant problems or discrepancies, and sufficient calibration and quality control
782 information to verify that the method was within control limits at the time that the samples were
783 analyzed. The case narrative will also include a discussion of any corrective action taken to
784 prevent the reoccurrence of similar problems or discrepancies.

785

786 **C.2 REPORTS TO MANAGEMENT**

787

788 The USGS-Columbia Project Manager will provide written quarterly reports to the USEPA
789 Project Manager. These reports will include the project status, any quality, budget, schedule, or
790 scope changes, and any quality issues that may affect the integrity of the project. USGS-
791 Columbia will also provide the USEPA Project Manager with periodic written or verbal progress
792 summaries (e.g., by email or phone calls or by conference calls organized by USEPA).

793

794 **SECTION D: DATA VALIDATION AND USABILITY**

795

796 **D.1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS**

797

798 Data will be reviewed by USGS prior to release, and data validation will be completed by CH₂M
799 Hill on behalf of USEPA. During the USGS data review process, the criteria established in
800 Section A.7 and Tables 8 and 10 will be used to evaluate the data. When the reviews identify
801 suspect data, that data will be investigated to establish whether it reflects true conditions or an
802 error. The investigation will be documented. If the data value is determined to be in error, the
803 source of the error will be investigated, the correct value established if possible, and the
804 erroneous value replaced with the correct value. If the investigation concludes that the data are
805 suspect (possibly in error) but a correct value cannot be determined, the data will be flagged to
806 indicate its suspect status. This process will determine whether the data can be accepted, rejected
807 or qualified.

808

809 A Data validation report will be completed by CH₂M Hill under a separate contract to USEPA.
810 Data will be validated and data qualifiers will be assigned according to the USEPA National
811 Functional Guidelines for Inorganic Data Review (USEPA 2004), with modifications made to
812 accommodate the methods that will be used for this project.

813

814 **D.2 VALIDATION AND VERIFICATION METHODS**

815

816 At USGS, a series of reviews by technical personnel will be implemented to ensure that the data
817 generated for this project meets the data quality objectives and indicators. These reviews will
818 include the following:

819

- 820 • Data will be reviewed by laboratory personnel at the end of each working day to ensure
821 that toxicity testing and analytical activities are completely and adequately
822 documented;

- 823 • About 10% of all calculations performed manually will be checked for accuracy.
824 Checking will be performed by qualified persons who did not participate in
825 performing the calculations; and,
- 826 • A 100% verification of data entry into spreadsheets will be performed. The staff
827 member performing the verification will assure correct entry into the software by
828 comparing data with the hard copy of the data listing. If errors are discovered, the
829 errors will be corrected and a new data listing will be generated.

830
831 Chemistry data validation by USEPA will consist of a review of initial calibrations and
832 calibration checks and all results for QC samples and procedures (Level 3 validation). Ten
833 percent of the data will receive full validation (Level 4) to verify the accuracy of calculations and
834 transcriptions. Additional full validation will be completed if errors are found to the extent
835 required to ensure an accurate database. Toxicity test data will be validated based on test
836 acceptability criteria (e.g., temperature, dissolved oxygen, pH, negative controls; Table 10),
837 precision (measurement duplicates), and completeness. Procedures will be compared to
838 protocols, and all manual data manipulations and transcriptions will be reviewed for accuracy.

839

840 **D.3 RECONCILIATION AND USER REQUIREMENTS**

841

842 In advance of completing the USGS-Columbia final report, full data packages of draft data will
843 be provided by USGS-Columbia to USEPA for all chemical and biological measurements.
844 CH₂M Hill on the behalf of USEPA will develop a data validation report based on these data
845 packages. The USGS-Columbia will then use validated data as summarized in the USEPA data
846 validation report in the preparation of the USGS-Columbia final report. See Section A10. for
847 additional detail. Any data that do not comply with the data quality objectives and indicators
848 identified in Section A.7 will be flagged and discussed in the final report. Any limitations on the
849 use of the data will also be reported.

850

851 **SECTION E: REFERENCES CITED**

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921

922 **Attachment 1. USGS-Columbia Data Rejection Criteria and Corrective Action Procedures**
923 **for Elemental Analyses (SOP P.239 dated 09/14/07)**
924

925 General: Elemental analyses are to be conducted with quality control measurements in a
926 consistent manner in order to assure the quality of the results. The CERC generally follows
927 quality control guidelines established by the USEPA for elemental analyses. This document
928 describes the acceptance/rejection criteria for various quality control parameters referred to in
929 SOP P.184 as well as the corrective action measures to be followed for data not meeting criteria.
930 The criteria outlined are considered targets rather than absolutes. In some instances, data with
931 associated QC samples that do not meet all criteria may only need to be "flagged" rather than
932 completely rejected. There are no P.A.R.T. updates for this SOP.
933

934 Safety: This SOP must be conducted in accordance with the CERC Safety and Chemical
935 Hygiene Plan. Supervisors are responsible for all safety training involved with this procedure.
936

937 1. Stocks and Standards

938 If it is discovered that a block of samples was analyzed with standards from stocks that were
939 considered out-of-date or expired, the samples must be re-analyzed with new standards.
940

941 2. Calibration/Calibration Verification

942 Calibration standards (minimum of 3) not used for calibration because of a particular instrument
943 configuration must be analyzed after calibration and must be within 5% of true values.
944 Similarly, the calibration verification solution (either from NIST stock or a stock from
945 independent source) must be within 10% of the expected value. If this target is not met, the
946 check solution should first be re-analyzed. If it still fails, the calibration must be repeated and
947 any samples analyzed after the previous acceptable calibration verification must be re-analyzed.
948 If recalibration fails to correct the discrepancy, new standards must be prepared.
949

950 3. Blanks/Blank Verification/Blank Correction

951 Three preparation blanks will be analyzed to provide the measurements necessary for the method
952 detection limit and blank correction. Results for blanks should be less than 10 times the average
953 of the 3 most recent IDLs, otherwise flagging and possibly re-preparation of all samples in the
954 block will be required. Rejection of a data block based on unusual sample blanks is dependent on
955 the level of analyte in samples and the level of the study. Generally, unless a litigation-sensitive
956 case is involved, only the samples that are less than ten times the highest blank will need to be
957 re-prepared. For blank verification analyses, rejection criteria will be based on control charts and
958 corrective action will consist of flagging of relevant data or possibly, re-analysis.
959

960 4. Limit of Detection

961 The method detection limit (MDL) is calculated by pooling the standard deviations of the
962 preparation blanks and a low-level sample or standard, and multiplying by 3.3. Calculated in
963 this manner, uncertainty in measuring an actual sample and correcting for reagent or preparation

964 blanks is accounted for. Criteria for flagging or rejection of sample data based on an abnormal
965 MDL would be based ultimately on control charting procedures. However, MDL's are highly
966 dependent on the sample matrix and the analytical method. Currently, insufficient data exists to
967 allow for control charting of most analyte/matrix/method combinations. Therefore, a target MDL
968 must be outlined and subsequently evaluated for each study based on the data requirements of
969 the principal investigator. Otherwise, rejection criteria will be based on whether the measured
970 MDL for a study is greater 3 standard deviations from historical data for the matrix. In general,
971 MDL criteria will be established for four (4) matrices: water, soil/sediment, plant, and biological
972 tissue. In the event that the measured MDL fails to meet the target, the analyses required for the
973 determination of the MDL will be repeated. If the MDL still fails the criteria, it must be
974 determined if the failure is caused by high preparation blanks or high variation for the analysis of
975 the low sample. If the failure is due to high variation from the analysis, the variable instrument
976 performance must be corrected and all sample analyses must be repeated. If the failure is due to
977 abnormal preparation blanks, the sample block must be re-digested and all samples re-analyzed.
978

979 5. Post-Digestion Analysis Spike

980 At least one analysis (post-digestion) spike will be conducted per ten (10) samples of each
981 matrix as a measurement of signal suppression or enhancement caused by sample matrix. For
982 each matrix, the average recovery of post-digestion spikes must be within 10% of 100; however,
983 no individual recovery should be more than 20% in error. If re-analysis for an individual sample
984 analysis spike does not meet the latter criteria, or if the average recovery criteria is not met, all
985 samples of a similar matrix must be analyzed by the method of standard additions.
986

987 6. Method Precision

988 The estimate for overall method precision is based on triplicate digestion and analysis and a
989 computation of the percent relative standard deviation (%RSD). In some cases a duplicate
990 analysis may be substituted and the relative percent difference (RPD) is evaluated. Targets for
991 RSD or RPD are $\pm 10\%$ for water and $\pm 20\%$ for other matrices. These targets are valid only if the
992 replicated sample has a concentration at least 10X the MDL. For some analytes the acceptable
993 limits of precision may be poorer. Ultimately, control charting methods will be used.
994

995 7. Reference Materials

996 In general, the acceptance criteria for reference materials is the certified range extended by
997 $\pm 10\%$ of the certified mean. Recovery criteria are valid only if the reference material analyzed
998 contains the analyte at a concentration at least 10X the MDL. A common exception is the case of
999 soil/sediment samples that are digested with partial extraction procedures or procedures that
1000 produce "acid extractable" or "total recoverable" metal concentrations. Certified reference
1001 values may not be applicable in this situation because certified ranges are usually based on
1002 "total" digestion procedures.
1003

1004 8. Pre-digestion Spikes

1005 The target criteria for pre-digestion spikes is for the average recovery for all spikes to be $100 \pm$
1006 20% , and that no individual spike recovery be more than 50% in error. Two conditions must be
1007 met in order for individual spiked samples to be judged by this criteria: (1) the spike-to-sample
1008 "background" ratio is at least 0.1 for water and 0.5 for sediment or tissue and (2) the spike to
1009 sample background standard deviation is at least 10. If a spiked sample meets these two
1010 conditions but fails the acceptance criteria, it should be re-analyzed. If criteria are still not met,
1011 the sample spike will be flagged. A failure of more than one spiked sample per analyte will
1012 normally require all samples in the block to be re-digested and re-analyzed, but this action may
1013 depend on the number of spikes and the performance of other QC parameters.

1014

1015 9. Duplicate Readings

1016 Reproducibility of duplicate readings should generally be within $\pm 10\%$ except at concentrations
1017 that are $\leq 20X$ of the IDL. For concentrations between $10X$ and $20X$ the IDL, agreement should
1018 be within $\pm 20\%$. No criteria are stated for values less than $10X$ the IDL. For atomic absorption
1019 measurements, these criteria translate roughly to $\pm 10\%$ for readings greater than 0.020
1020 absorbance units and $\pm 20\%$ for readings from 0.010 to 0.020 absorbance units. Each sample
1021 having duplicate readings outside these criteria must be flagged and possibly rerun.

1022

1023 10. Background/Spectral Interference Check

1024 Before completing analysis by graphite furnace atomic absorption, the absorbance of 2-3
1025 representative samples will be measured in the "BG" only mode to check for abnormally high
1026 background absorption. For flame emission measurements, spectral background will be checked
1027 by measuring a few samples just off the analytical wavelength. For ICP/ICP-MS
1028 determinations, spectral interferences from samples can be checked by one of three methods,
1029 whichever is most suitable:

1030

1031 (a) A representative sample shall be analyzed at a secondary wavelength/mass and compared to
1032 results from primary line/mass.

1033

1034 (b) A representative sample shall be diluted by at least five-fold, reanalyzed, and compared to
1035 original (undiluted) result.

1036

1037 (c) For determinations with known interferences, a check solution containing the analyte and
1038 interfering element(s) shall be analyzed.

1039

1040 Results for (a), (b), or (c), should be within 10% of the undiluted or true value. If not,
1041 troubleshooting and elimination of the spectral interference must be documented or an alternate
1042 method of analysis performed.

1043

1044 11. Standard Additions

1045 Samples determined by the method of additions must be analyzed by the addition of a blank and
1046 spikes of " $0.5x$ " and " x " where " x " is equal to an analyte concentration yielding from 0.050 to

1047 0.100 absorbance units and the sample concentration is < "x". The correlation coefficient for
1048 each regression plot must be 0.995 or greater, otherwise the sample must be rerun. If the
1049 calculated sample concentration is > "x", it must be diluted and rerun. The calibration
1050 verification solution must also be analyzed by additions and the result be within + 10% of true
1051 value before samples can be run.

1052

1053 12. Procedural Errors/Deviations

1054 Occasionally, a procedural error may cause a suspicious result during sample preparation or
1055 analysis. For example, a technician/analyst may suspect that two samples have been incorrectly
1056 labeled due to sample appearance or an analysis of several samples from the same site may
1057 reveal one sample with quite different concentrations of one or more elements. The corrective
1058 action employed depends upon the stage at which the suspicion arose:

1059

1060 Chemical preparation: Procedural errors at the chemical preparation stage, such as accidental
1061 spilling or dropping, digestion losses, wrong spiking or dilution, or possible mis-labeling should
1062 be documented by the technician/analyst and brought to the immediate attention of the
1063 supervisor, who will recommend the proper correction based on extent of loss or degree of error.
1064 Corrective action may range from continuation of process to complete sample re-preparation.

1065

1066 Instrumental analysis: An analyst may decide to reanalyze a sample for various reasons, which
1067 include large variation in duplicate analyses, off-scale concentrations, unusual concentration
1068 compared to other samples, etc. The corrective action for reanalysis at this stage involves written
1069 documentation of the reason(s) for rejecting the first analysis on the instrument printout and any
1070 relevant worksheets. All "reshoots" must be labeled as such on instrumental analysis worksheets.

1071

1072 13. Data Review

1073 Data acquired relative to sample processing, including log-in and inventory, non-chemical
1074 preparation, chemical preparation, and instrumental analysis will be reviewed by the Team
1075 Leader. Specific items reviewed are outlined on "Progressive Sample Flow Checklists" for each
1076 phase of sample processing. Errors are documented as the review process occurs and become
1077 part of the collective data. Emphasis is placed on checking all data points (100% review) where
1078 manual transmissions occur, i.e., from instrument printouts to worksheets, and from worksheets
1079 to spreadsheets or to final reports. Corrective action involves having the technician/analyst
1080 make necessary error corrections as determined by Team Leader. Error corrections are then
1081 verified by Team Leader.

1082

1083 An overall evaluation of the final data and quality assurance will be the responsibility of the
1084 Team Leader and the Principal Investigator. On a rare occasion, some final data may appear
1085 suspicious for reasons other than the quality control parameters previously listed. For example,
1086 samples from the same site may include one sample with a concentration that is quite different
1087 for one or more analytes. In such cases, an incorrect decimal point, dilution factor, a mislabeled
1088 sample or other technical errors must be evaluated. Corrective action will include a re-evaluation

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1089 of all sample labels and a re-check for a possible transcription error. Further action will include
1090 the re-analysis of the sample digestate or the original sample, if available. Strict documentation
1091 will be maintained of all re-analyses and any data deemed to be in error (with justification for
1092 disregarding). Normally, any value that is considerably greater than all other values of a study is
1093 a candidate for re-analysis.

Table 1. Project organization and roles and responsibilities.

Personnel	Role and responsibility
Ed Little and Chris Ingersoll	USGS-Columbia Co-Project Managers: Provide overall direction of the project for USGS-Columbia (Little Tasks 1 and 2; Ingersoll Tasks 3, 4, and 5). Ensure that all tasks are accomplished in a timely manner and within project budget. Assign qualified staff to the project. Develop planning documents and budget. Have direct contact with the USGS-Columbia QA Officer and EPA.
Paul Heine	USGS-Columbia QA Officer: Provides QA support at all levels, in such areas as QAPP development and revision, resolving QA problems, document review, and data validation. Provides independent oversight to verify that project activities are being conducted in a manner consistent with requirements identified in this QAPP.
Ning Wang and Robin Calfee	USGS Co-Principal Investigators: Lead investigators for toxicity testing and data analysis (Calfee: Task 2; Wang: Task 3). Oversee day-to-day activities in the laboratory. Responsible for laboratory facilities and test equipment and providing direction to staff. Ensure all laboratory records related to the test are completed and reviewed for completeness and accuracy. Work with USGS-Columbia Project Managers to coordinate schedule. Chris Mebane will provide assistance in Tasks 4 and 5 regarding the interpretation of toxicity data relative to water quality and relative to the biotic ligand model.
Ryan Warbritton	USGS Co-Principal Investigator: Primary lead for collection and culture of fish in Task 1. Oversees day-to-day activities in the culture facility. Is responsible for laboratory facilities and test equipment and providing direction to staff. Ensures that all laboratory records related to the test are completed and reviewed for completeness and accuracy. Works with USGS-Columbia Project Managers to coordinate schedule.
Bill Brumbaugh	USGS Co-Principal Investigator: Lead investigators for chemical analyses (Tasks 2 and 3). Oversee day-to-day activities in the laboratory. Responsible for laboratory facilities and test equipment and providing direction to staff. Ensure all laboratory records related to the test are completed and reviewed for completeness and accuracy. Work with USGS-Columbia Project Managers to coordinate schedule.
Helen Bottcher	USEPA Project Manager: Has overall responsibility of project. Works with the USGS-Columbia Project Managers to ensure that project tasks are being met.
Gina Grepo-Grove	USEPA QA Officer: Reviews the QAPP to ensure that the contents are adequate for the project scope and meet the requirements of EPA. Review draft data including the Quality Assurance reports and provide USGS with a data validation report based on the review of the draft data. A Data validation report will be completed by CH ₂ M Hill under a separate contract to USEPA. Data will be validated and data qualifiers will be assigned according to the USEPA National Functional Guidelines for Inorganic Data Review, with modifications made to accommodate the methods that will be used for this project (Section D1).

Table 2. Conditions for culturing and handling white sturgeon or with rainbow trout to be used in water toxicity tests (adapted from ASTM 2009a,b).

Parameter	Conditions
1. Source of organisms	White sturgeon (<i>Acipenser transmontanus</i>): State of Washington Columbia Basin Hatchery Rainbow trout (<i>Oncorhynchus mykiss</i>): Ennis National Fish Hatchery, Ennis Montana
2. Test type:	Not applicable
3. Temperature (°C):	Sturgeon: 15°C Trout: 12°C Fish will be acclimated to exposure water for at least 48 h before the start of an exposure (see Item 18 below dealing with acclimation)
4. Light quality:	Ambient laboratory light
5. Light intensity (lux):	About 200
6. Photoperiod (light:dark):	16:8
7. Hatching Tanks (L):	Sturgeon: Modified MacDonald hatching jars (7-cm tall by 2-cm diameter). Sturgeon eggs will circulate better in the jars if there are more eggs, to a point. Therefore 3,000 to 6,000 sturgeon eggs will be placed in each hatching jar (about 6 to 8 hatching jars total for sturgeon). Trout: Heath incubator and trays. MacDonald hatching jars and are 6" diameter and 16" tall.
8. Holding Tanks (L):	Sturgeon: 1,850-L rectangular fiberglass tank (46-cm tall x 84-cm wide x 483-cm long) Trout: 400-L round polyethylene tanks (91-cm diameter x 61-cm tall)
9. Water Flow:	Water flow through hatching jars and incubator will provide adequate flow over the unhatched or hatching eggs and will be adjusted daily as necessary. Water flow in the holding containers will be adequate to provide several volume additions/day
10. Organisms/chamber:	Not applicable
11. Loading:	Not applicable
12. Replicates:	Not applicable
13. Duration (d):	Not applicable
14. Age of test organisms:	Sturgeon: Received as newly fertilized eggs (about <3-days post fertilization) Trout: Received as eyed eggs. Sturgeon or trout will be obtained by spawning at least 2 female fish and 2 male fish.
15. Feeding:	Sturgeon: Food will be introduced about a week before the start of exogenous feeding. Fed newly hatch brine shrimp <i>ad libitum</i> several times daily as a first food transitioning to chopped and then whole live oligochaetes (<i>Lumbriculus variegatus</i>) as sturgeon are able to eat. Other commercially available diets may be offered as necessary. Trout: Food will be introduced about a week before the start of exogenous feeding. Fed newly hatch brine shrimp <i>ad libitum</i> several times daily as a first food transitioning to commercially available trout diet and transition to chopped and then whole live oligochaetes (<i>Lumbriculus variegatus</i>) as trout are able to eat. Nutritional analyses of a batch of <i>Lumbriculus variegatus</i> will be performed on two composit sample collect during the sturgeon and trout chronic exposures (percent moisture, protein, lipid, carbohydrate, ash, amino acid and fatty acid profiles by Eurofins Scientific, Memphis TN).

Table 2. Conditions for culturing and handling white sturgeon or with rainbow trout to be used in water toxicity tests (adapted from ASTM 2009a,b).

Parameter	Conditions
16. Cleaning/Maintenance:	Siphoned and walls of holding tank wiped down as needed. Hatching jars/holding tanks will be checked daily and dead eggs/fish will be removed at least 3 times/week or as needed.
17. Culture water:	Eggs will be initially held in diluted well water (about 100 mg/L hardness as CaCO ₃ and pH 8.2) until hatching (hence, 1 dph fish used to start exposures will not need to be acclimated to the exposure water). Hatched fish will be held in well water (about 300 mg/L hardness as CaCO ₃ and pH 8.2) before being acclimated to 100 mg/L hardness test water at the start of exposures (see Item 18 below on Acclimation). Temperatures maintained by passing water through stainless-steel heat exchangers located in insulated water baths with Frigid Unit chillers.
18. Acclimation	Pipets will be used to transfer hatched fish from the cultures to acclimation containers or from acclimation containers to the exposure chambers (fish will not be in contact with the atmosphere). Fish will be acclimated for at least 48 hours to test water and test temperature under static renewal conditions (water additions 1 to 2 times/day) with aeration and the temperature change will not exceed about a 3°C within 12 h. Fish used in acute tests will not be fed and fish used in chronic tests may be provided with live <i>Artemia</i> or <i>Lumbriculus</i> (e.g., for exogenous feeding fish at the start of Chronic biphasic stage 2 testing).
19. Dilution series:	Not applicable
20. Chemicals:	Not applicable
21. Water quality:	Temperature daily. Other water quality parameters (dissolved oxygen, pH, conductivity, alkalinity, hardness, and ammonia) will be measured at least weekly.
22. Aeration:	Sturgeon: Light aeration in tank after hatching Trout: Light to medium aeration in holding tank after swim-up
23. Endpoints:	Not applicable
24. Test acceptability:	Not applicable

Table 3. Acute toxicity water-only studies for the white sturgeon (WS) and rainbow trout (RT). Gray blocks represent life stages that have been previously tested by USGS or by the University of Saskatchewan.

Days Post hatch (DPH)		Cadmium				Copper				Zinc			
Start	End	WS		RT		WS		RT		WS		RT	
		UofS	USGS	UofS	USGS	UofS	USGS	UofS	USGS	UofS	USGS	UofS	USGS
0	7	2008	2010**		2010**	2008	2010**		2010**	2008	2010**		2010**
8	14	○				○		○		○			
15	21		2010		2010	2010*	2010	2010*	2010		2010		2010
22	28						○						
29	35		2010x		2010x		2010x		2010x		2010x		2010x
36	42		○			○	○	○			○		
43	49		2010		2010	2010*	2010	2010*	2010		2010		2010
50	56												
57	63		2010		2010		2010		2010		2010		2010
64	70												
71	77		2010		2010		2010		2010		2010		2010
78	84												
85	91												
91	98		2010		2010	○	○		2010		2010		2010
99	105												
106	112												
113	119						○160 day						
							○450 day						

Notes:

○ Unpublished data

US Geological Survey (USGS)

University of Saskatchewan (U of S)

2008 2008 chronic toxicity studies conducted by U of S

2010* 2010 acute toxicity studies to be conducted by USGS and by U of S

2010 2010 acute toxicity studies to be conducted by USGS

2010** 2010 chronic toxicity studies conducted by USGS

2010x Change from February 23, 2010 draft

Table 4. Conditions for conducting acute or chronic water-only toxicity tests with white sturgeon and rainbow trout (adapted from ASTM 2009a,b,c).

Parameter	Conditions
1. Species	White sturgeon (<i>Acipenser transmontanus</i>) Rainbow trout (<i>Oncorhynchus mykiss</i>)
2. Test type:	Water-only exposures in intermittent proportional diluters with toxicant stock solutions delivered with each cycle of the diluters by Hamilton syringe pumps (Hamilton, Reno, NV, USA; Wang et al. 2007). Sturgeon testing to start in about early July 2010 and trout testing to start in the fall of 2010 (timing dependant on availability of fish).
3. Temperature (°C):	15 (temperatures maintained by passing water through stainless-steel heat exchangers located in insulated water baths with Frigid Unit chillers)
4. Light quality:	Ambient laboratory light
5. Light intensity (lux):	About 200
6. Photoperiod (light:dark):	16:8
7. Test chamber size (L):	Acute Test: up to 9.5 Chronic Tests: 9.5 (chronic tests will have 5 medium-size pieces of gravel (1- to 2-cm diameter) placed in each chamber up to the swim-up life Stage (to reduce activity of fish through the hiding Stage; Ed Little, USGS-Columbia, unpublished data)
8. Test solution volume (L):	Acute tests: up to 7 Chronic tests: 7
9. Water addition (L):	Acute Test: 0.5/chamber/30 min (3.4 volume additions/day) Chronic Tests: 0.5/chamber/30 min (3.4 volume additions/day). Water addition may be increased or number of fish may be reduced in a chamber to accommodate increased mass of fish at the start of the acute tests or during the chronic tests (see Item #11 below on "Loading")
10. Organisms/chamber:	Acute Test: 10 (minimum) Chronic Biphaseic Stage 1 Exposure: 20 (minimum) Chronic Biphaseic Stage 2 Exposure: 10 Chronic Continuous Test: 20 (minimum; thinned to 10 by about Day 28). Thinning of fish will be done impartially, by no more than two individuals technicians, in order to remove representative fish (size and behavior) from an exposure chamber (ASTM 2009b).
11. Loading:	<0.5 g fish/L of test solution passing through the test chamber over 24 h and <5 g fish/L in the test chamber at any given time Note: At the end of a 28-d feeding study started with 30 day post hatch (dph) sturgeon in 2009, loading was 2.1 g fish/L with 0.63 g fish/L over 24 h (1.5 g/fish x 10 fish in 7 L of water; Ning Wang, USGS-Columbia, unpublished data)
12. Replicates:	4 in the Chronic Biphaseic Stage 1 and Stage 2 Exposures (Stage 1 and Stage 2 conducted sequentially) 4 in the Chronic Continuous Test conducted concurrently with the Chronic Biphaseic Stage 1 and Stage 2 Exposures

Table 4. Conditions for conducting acute or chronic water-only toxicity tests with white sturgeon and rainbow trout (adapted from ASTM 2009a,b,c).

Parameter	Conditions
13. Duration (days):	<p>Acute test: 4</p> <p>Chronic continuous Test: 56 (starting with about 1 dph fish)</p> <p>Chronic Biphasic Stage 1 Exposure: 21 (starting with about 1 dph fish; continued until about 50% of the fish begin to swim up). Note: The Chronic Biphasic Stage 1 Exposures may be continued for more than 21 days if control survival has not dropped below about 80%.</p> <p>Chronic Biphasic Stage 2 Exposure: 28 (starting with about 28 dph exogenous-feeding fish)</p>
14. Age of test organisms:	<p>About 20 fish will be destructively sampled at the start of each acute or chronic exposure for measurement of length and dry weight.</p> <p>Acute test: See Table 3 for ages of organisms to be tested</p> <p>Chronic Continuous Test: About 1 dph fish</p> <p>Chronic Biphasic Stage 1 Exposure (21-d duration): About 1 dph</p> <p>Chronic Biphasic Stage 2 Exposure (28-d duration): About 28 dph (at exogenous feeding)</p>
15. Feeding:	<p>Acute test: None</p> <p>Chronic Biphasic Stage 1 Exposure and Chronic Continuous Test: No feeding up to about Day 14. At about Day 14, brine shrimp (<i>Artemia</i>) will be introduced (e.g., before the onset of exogenous feeding).</p> <p>Chronic Biphasic Stage 2 Exposure and Chronic Continuous Test (at exogenous feeding): <i>Ad libitum</i> twice daily starting with brine shrimp (<i>Artemia</i>) for about 1 week with transition to live oligochaetes (<i>Lumbriculus variegatus</i> (monitoring daily food consumption))</p>
16. Chamber cleaning:	At least once daily
17. Test water:	Diluted well water: 100 mg/L hardness as CaCO ₃ , pH 8.1, alkalinity 92 mg/L, conductivity 220 µS/cm, DOC 0.5 mg C/L (24 mg Ca/L, 9 mg Mg/L, 8 mg Na/L, 0.8 mg K/L, 10 mg Cl/L, 15 mg SO ₄ /L (Besser et al. 2007)
18. Dilution series:	<p>50% dilution series (control and 5 test concentrations)</p> <p>Acute: Concentrations adjusted by life stage (potential ranges: 1 to 48 µg Cu/L; 6 to 40 µg Cd/L, 30 to 1500 µg Zn/L)</p> <p>Chronic: See Table 6 for the copper, cadmium, zinc, and lead exposure concentrations</p>
19. Chemicals:	<p>Copper (II) sulfate pentahydrate (Chemical Abstracts Service (CAS) number 7758-99-8), cadmium chloride hemi-pentahydrate (CAS number 7790-78-5), zinc chloride (CAS number 7646-85-7) and lead nitrate (CAS number 10099-74-8) from Sigma-Aldrich. See Table 7, 8 for the frequency of analyses of metals (filtered to <0.45 µm). Toxicant stock solutions prepared in deionized water.</p>

Table 4. Conditions for conducting acute or chronic water-only toxicity tests with white sturgeon and rainbow trout (adapted from ASTM 2009a,b,c).

Parameter	Conditions
20. Water quality:	Temperature (daily), conductivity, dissolved oxygen, hardness, alkalinity, pH, ammonia, major cations, major anions, dissolved organic carbon (see approximate schedule in Tables 5 and 7). Particulate organic carbon (POC) will be monitored periodically during the chronic exposures on unfiltered samples (i.e., concurrently sampled with at least 10% of the DOC samples; Table 7). Frequency for measuring water quality will be increased if substantial changes are observed during exposures. Alternatively, the frequency of measured DOC, cation, anions may be decreased if there are not substantial differences initially observed across treatments in the acute or chronic exposures (any substantial decrease in the frequency of measuring water quality will be discussed with USEPA in advance of making this change).
21. Aeration:	None unless dissolved oxygen <4 mg/L
22. Endpoints:	<p>Acute and Chronic: Survival, behavior (daily)</p> <p>Chronic: Growth at end of Biphasic 28-day exposure and chronic continuous exposure (average total length and average wet weight) and biomass. Wet weight of fish by replicate will be determined by gently blotting fish on a dry paper towel before weighing the fish. Fish will be preserved in 10% formalin for morphological measurements (e.g., external gross pathology using methods developed by the U of S; Markus Hecker, personal communication) and for measurement of length of individual fish. Dry weight of fish by replicate will be determined after morphological and length measurements have been completed by drying surviving fish to a constant weight at 60°C (e.g., for 24 h of drying).</p> <p>Behavior: Includes endpoints such as change in fish: location in aquaria, loss of equilibrium, feeding, activity, coloration, or respiration (see Figure 1 in ASTM 2009c). Photos or videos may be created to qualitatively document potential morphological or behavioral responses.</p>
23. Test acceptability:	<p>Acute Test: 90% control survival</p> <p>Chronic Continuous Test: 64% control survival (starting with about 1 dph fish)</p> <p>Chronic Biphasic Exposures: 80% control survival during Stage 1 Exposure (starting with about 1 dph fish) and 80% control survival during Stage 2 Exposure (starting with about 28 dph exogenous-feeding fish)</p> <p>See Table 10 for additional detail.</p>

Table 5. General activity schedule for conducting acute or chronic toxicity tests with white sturgeon and rainbow trout (adapted from ASTM 2009a,b,c).

Day	Activity
-14 to 0	<ul style="list-style-type: none"> ●Receive and culture eggs ●Calibrate diluters
-2	<ul style="list-style-type: none"> ●Acclimate fish to test water and temperature
0 (and during tests)	<ul style="list-style-type: none"> ●Start: Introduce acclimated fish to exposure chambers. Measure length, wet weight, and dry weight on a subset of about 20 fish. ●Daily: Check function of diluters, record fish survival and behavior (Table 4), remove and dispose of dead fish, feed fish, siphon chambers as required (Table 4) ●Acute Test: Measure routine water quality in at least 3 treatments and sample water for dissolved metals in all 6 treatments (Day 0 and Day 4; Table 7) and sample water for dissolved organic carbon, major cations and major anions in at least 2 treatments at the start of the exposures (Table 7) ●Chronic Tests: Measure routine water quality in at least 3 treatments and sample water for dissolved metals in all 6 treatments (weekly; Table 7) and sample water for dissolved organic carbon, major cations and major anions in at least 2 treatments (about weekly; Table 7)
4	<ul style="list-style-type: none"> ●Acute Test: End exposure by recording fish survival, behavior, and water quality measurements (see Day 0), euthanize live fish (in MS222), dispose of fish
21	<ul style="list-style-type: none"> ●Chronic Biphasic Stage 1 21-day Exposure: (starting with about 1 day post hatch (dph) fish): End exposure by recording survival, behavior and water quality measurements, euthanize live fish (in MS222), dispose of fish. Exposures may be continued for more than 21 days if control survival has not dropped below about 80%.
26	<ul style="list-style-type: none"> ●Chronic Biphasic Stage 2 28-day Exposure: Acclimate fish to test water and temperature (timing of this step will be dependant on the onset of exogenous feeding)
28	<ul style="list-style-type: none"> ●Start Chronic Biphasic Stage 2 28-day Exposure: 28 (starting with about 28 dph exogenous feeding fish) by introducing acclimated fish to the exposure chambers ●Daily Chronic Biphasic Stage 2 28-day Exposure: Check function of diluters, record fish survival and behavior (Table 4), remove and dispose of dead fish, feed fish, siphon chambers as required (Table 4) ●Chronic Biphasic Stage 2 28-day Exposure: Measure routine water quality in at least 3 treatments and sample water for dissolved metals in all 6 treatments (weekly; Table 7) and sample water for dissolved organic carbon, major cations and major anions in at least 2 treatments (about weekly; Table 7)
56	<ul style="list-style-type: none"> ●Chronic Biphasic Stage 2 Exposure and Chronic Continuous Exposure: Sample surviving fish for individual measurements of length and wet weight; preserve fish in 10% formalin for potential morphological analyses.

Table 6. Historic toxicity data and selected exposure concentrations for conducting chronic water-only toxicity test with white sturgeon (WS) and rainbow trout (RT) by USGS-Columbia in 2010 (dph = days post hatch). **DISCLAIMER: PROVISIONAL STURGEON TOXICITY DATA FROM THE U OF S OR FROM THE USGS-COLUMBIA ARE SUBJECT TO CHANGE; HENCE, THESE DATA CANNOT BE CITED, QUOTED, OR DISTRIBUTED. THESE STURGEON TOXICITY DATA ARE ONLY BEING USED FOR THE PURPOSE OF ESTABLISHING METAL EXPOSURE CONCENTRATIONS IN THE ACUTE AND CHRONIC STURGEON EXPOSURES TO BE CONDUCTED BY USGS-COLUMBIA IN 2010.**

Markus Hecker, U of S (hardness 70 as CaCO ₃ and DOC 2.0 mg C/L; unpublished provisional data) ^A	Species	Measured LC20 (µg/L)	Life stage	Duration (days)	Hardness (as CaCO ₃)	DOC (mg C/L)	Predicted LC20 (µg/L) (@ hardness 100, pH 8.2, DOC 0.5 mg C/L)	Predictive tool
Copper	WS	6.3	Egg to juvenile (58 dph)	66	70	2.0	1.0	BLM ^B
Zinc	WS	107	Egg to juvenile (58 dph)	66	70	2.0	90	BLM ^C
Cadmium	WS	1.2	Egg to juvenile (58 dph)	66	70	2.0	1.5	BLM ^C
Markus Hecker, U of S (hardness 70 as CaCO ₃ and DOC 2.0 mg C/L; unpublished provisional data) ^A	Species	Measured LC50 (µg/L)	Most sensitive life stage (days post hatch)	Duration (days)	Hardness (as CaCO ₃)	DOC (mg C/L)	Predicted LC50 (µg/L) (@ hardness 100 and DOC 0.5 mg C/L)	Predictive tool
Copper	WS	17	8 dph	4	70	2.0	11	BLM ^B
Zinc	WS	145	8 dph	4	70	2.0	155	BLM ^C
Cadmium	WS	18	8 dph	4	70	2.0	22	BLM ^C
Ed Little, USGS-Columbia (hardness 100 as CaCO ₃ , pH 8.2, DOC 0.5 mg C/L; unpublished provisional data) ^A	Species	Measured Cu LC50 Zn or Cd EC50 (µg/L)	Most sensitive life stage (days post hatch)	Duration (days)	Hardness (as CaCO ₃)	DOC (mg C/L)	Predicted LC50 (µg/L) (@ hardness 100 and DOC 0.5 mg C/L)	Predictive tool
Copper	WS	7.2	35	4	100	0.5	NA ^D	NA
Zinc	WS	51	30	4	100	0.5	NA	NA
Cadmium	WS	11	30	4	100	0.5	NA	NA
Besser et al. (2007; hardness 100 as CaCO ₃ , pH 8.2, DOC 0.5 mg C/L)	Species	Measured ChV (µg/L)	Life stage	Duration (days)	Hardness (as CaCO ₃)	DOC (mg C/L)	Predicted EC20 (µg/L) (@ hardness 100 and DOC 0.5 mg C/L)	Predictive tool
Copper	RT	40	Swim-up	28	100	0.5	NA ^D	NA
Zinc	RT	219	Swim-up	28	100	0.5	NA	NA
Cadmium	RT	1.9	Swim-up	28	100	0.5	NA	NA
Besser et al. (2007; hardness 100 as CaCO ₃ , pH 8.2, DOC 0.5 mg C/L)	Species	Measured LC50 (µg/L)	Most sensitive life stage (days post hatch)	Duration (days)	Hardness (as CaCO ₃)	DOC (mg C/L)	Predicted LC50 (µg/L) (@ hardness 100 and DOC 0.5 mg C/L)	Predictive tool
Copper	RT	58	Swim-up	4	100	0.5	NA ^D	NA
Zinc	RT	263	Swim-up	4	100	0.5	NA	NA
Cadmium	RT	3.7	Swim-up	4	100	0.5	NA	NA
USGS-Columbia nominal concentrations for 2010 chronic white sturgeon exposures (µg/L) ^E	Control	Low	Medium-low	Medium	Medium-high	High	USGS-Columbia Control water	
Copper	0	0.50	1.0	2.0	4.0	8.0	0.3 (Wang et al. 2010)	
Zinc	0	25	50	100	200	400	<5.0 (Besser et al. 2007)	
Cadmium	0	0.75	1.5	3.0	6.0	12	<0.04 (Besser et al. 2007)	
Lead ^F	0	5	10	20	40	80	<0.07 (Besser et al 2005)	
USGS-Columbia nominal concentrations for 2010 chronic rainbow trout exposures (µg/L) ^G	Control	Low	Medium-low	Medium	Medium-high	High	USGS-Columbia Control water	
Copper	0	10	20	40	80	160	0.3 (Wang et al. 2010)	
Zinc	0	50	100	200	400	800	<5.0 (Besser et al. 2007)	
Cadmium	0	0.75	1.5	3.0	6.0	12	<0.04 (Besser et al. 2007)	
Lead ^F	0	5	10	20	40	80	<0.07 (Besser et al 2005)	

^AProvisional data subject to change, cannot be cited, quoted, or distributed.

^BBLM (biotic ligand model) for copper (USEPA 2007)

^CBLM for zinc, cadmium, or lead (Robert Santore, Hydroqual Environmental Engineers and Scientists, East Syracuse, NY; unpublished)

^DNot applicable

^EConcentrations selected to bracket chronic effect concentrations for white sturgeon, hence effect concentrations for rainbow trout may be greater than or less than the selected ranges of exposure concentrations for white sturgeon.

^FConcentrations selected to bracket based on a proposed revised criterion for lead of 9.2 µg Pb/L (summarized Wang et al. 2010) and based on studies summarized by Mebane et al. (2008)).

^GConcentrations selected to bracket chronic effect concentrations for rainbow trout, hence effect concentrations for rainbow trout may be greater than or less than the selected ranges of exposure concentrations for white sturgeon.

Table 7. USGS-Columbia summary of approximate water and chemistry sampling for acute and chronic water-only toxicity test with white sturgeon and rainbow trout.

Task	Activity	Description	Design	Units
2a. Acute copper	Toxicity tests	Two species	2 species x 7 lifestages	14
	Water quality	Routine water quality	3 conc x 2 dates x 14 tests	84
		DOC	2 treatments x 14 tests	28
		Anions	2 treatments x 14 tests	28
		Cations	2 treatments x 14 tests	28
		Chemistry	Metal analyses	6 conc x 2 dates x 14 tests
2b. Acute cadmium	Toxicity tests	Two species	2 species x 7 lifestages	14
	Water quality	Routine water quality	3 conc x 2 dates x 14 tests	84
		DOC	2 treatments x 14 tests	28
		Anions	2 treatments x 14 tests	28
		Cations	2 treatments x 14 tests	28
		Chemistry	Metal analyses	6 conc x 2 dates x 14 tests
2c. Acute zinc	Toxicity tests	Two species	2 species x 7 lifestages	14
	Water quality	Routine water quality	3 conc x 2 dates x 14 tests	84
		DOC	2 treatments x 14 tests	28
		Anions	2 treatments x 14 tests	28
		Cations	2 treatments x 14 tests	28
		Chemistry	Metal analyses	6 conc x 2 dates x 14 tests
3a. Chronic copper exposure	Toxicity test	Two species	Biphasic and continuous for WS and RT	2
	Water quality	Routine water quality	3 conc x 8 dates x 2 species +20% cross check ^A	58
		DOC	3 conc x 8 dates x 2 species +20% cross check	58
		Anions	2 conc x 5 dates x 2 species +20% cross check	24
		Cations	2 conc x 5 dates x 2 species +20% cross check	24
		Chemistry	Metal analyses	6 conc x 8 dates x 2 species +20% cross check
3b. Chronic cadmium exposure	Toxicity test	Two species	Biphasic and continuous for WS and RT	2
	Water quality	Routine water quality	3 conc x 8 dates x 2 species +20% cross check ^A	58
		DOC	3 conc x 8 dates x 2 species +20% cross check	58
		Anions	2 conc x 5 dates x 2 species +20% cross check	24
		Cations	2 conc x 5 dates x 2 species +20% cross check	24
		Chemistry	Metal analyses	6 conc x 8 dates x 2 species +20% cross check
3c. Chronic lead exposure	Toxicity test	Two species	Biphasic and continuous for WS and RT	2
	Water quality	Routine water quality	3 conc x 8 dates x 2 species +20% cross check ^A	58
		DOC	3 conc x 8 dates x 2 species +20% cross check	58
		Anions	2 conc x 5 dates x 2 species +20% cross check	24
		Cations	2 conc x 5 dates x 2 species +20% cross check	24
		Chemistry	Metal analyses	6 conc x 8 dates x 2 species +20% cross check
3d. Chronic zinc exposure	Toxicity test	Two species	Biphasic and continuous for WS and RT	2
	Water quality	Routine water quality	3 conc x 8 dates x 2 species +20% cross check ^A	58
		DOC	3 conc x 8 dates x 2 species +20% cross check	58
		Anions	2 conc x 5 dates x 2 species +20% cross check	24
		Cations	2 conc x 5 dates x 2 species +20% cross check	24
		Chemistry	Metal analyses	6 conc x 8 dates x 2 species +20% cross check

^A20% cross checks across biphasic and continuous exposures within a species

Table 8. Data quality indicators for water quality samples and metal in water samples measured in toxicity tests. NA= Not applicable

Analyte	Method	Method detection limit	Target accuracy	Target precision (Relative Standard Deviation; %)	Target completeness (%)
Water Temperature	Orion 140 S-C-T Meter	About 0.2°C	±0.5°C	10 (except for POC at about 20)	90
Dissolved Oxygen	YSI 54a Meter and YSI 5739 Probe	About 0.5 mg/L	±0.5 mg/L		
Alkalinity	Orion EA940 Meter	About 5 mg/L	±10 mg/L		
Hardness	EDTA Titration	About 5 mg/L	±10 mg/L		
Conductivity	Orion 140 S-C-T Meter	About 10 µS/cm	±10 µS/cm		
pH	Orion EA940 Meter	NA	±0.1 unit		
Ammonia (NH ₃ ; mg/L)	Orion EA 940	About 0.1 mg/L	80-120%		
Dissolved Organic Carbon (DOC)	Technicon Colorimetry and USEPA 415.2	About 0.2 mg/L	80-120%		
Particulate Organic Carbon (POC)	Coulometrics Model 5010 (UIC Corporation, Joliet IL)	About 50 µg/L	80-120%		
Major Anions (nitrate+nitrite, sulfate, chloride, fluoride)	USEPA 300.0	About 0.5 mg/L	80-120%		
Major Cations (calcium, magnesium, potassium, sodium)	USEPA 200.7	About 1 mg/L	80-120%		
Cadmium	USEPA 1638 Brumbaugh et al. (2007)	0.05 µg/L	80-120		
Copper		0.05 µg/L			
Lead		0.05 µg/L			
Zinc		2 µg/L			

Table 9. Summary of responsibilities, containers, volume requirements, preservation, and holding times.

Medium	Analytical Laboratory	Analyte Group	Analytical Method (or the equivalent)	Container Material	Container Volume	Minimum Sample	Preservation Method	Holding Time
Exposure Water (filtered <0.45µm)	USGS-Columbia	Dissolved Metals	EPA 1638; Brumbaugh et al (2007)	LDPE bottle	30 mL	20 mL	0.16M HNO ₃	6 months
Exposure Water (unfiltered)	USGS-Columbia	Ammonia	Orion EA940 Meter	Glass	125 mL	125 mL	none	4 hr
	USGS-Columbia	pH	Orion EA940 Meter	<i>From same bottle listed above.</i>				
	USGS-Columbia	Temperature	Orion 140 S-C-T Meter	<i>From same bottle listed above.</i>				
	USGS-Columbia	Hardness	EDTA Titration	<i>From same bottle listed above.</i>				
	USGS-Columbia	Alkalinity	Orion EA940 Meter	<i>From same bottle listed above.</i>				
	USGS-Columbia	Conductivity	Orion 140 S-C-T Meter	<i>From same bottle listed above.</i>				
	USGS-Columbia	Dissolved oxygen	YSI 54a Meter & YSI 5739 Probe	<i>From same bottle listed above.</i>				
	USGS-Columbia	POC	Coulometrics Model 5010 (UIC Corporation, Joliet IL)	<i>From same bottle listed above.</i>				
Exposure Water (filtered <0.45µm)	LET Lab (Columbia, MO)	Major Cations	EPA 200.7	LDPE bottle	30 mL	15 mL	0.16M HNO ₃	6 months
	USGS-Columbia/USGS-Denver	Major Anions (other than nitrate+nitrite)	EPA 300.0	HDPE	10 mL	10 mL	4°C	28 d
	USGS-Columbia/USGS-Denver	Nitrate+nitrite	EPA 300.0	HDPE	10 mL	10 mL	4°C / pH 2 H ₂ SO ₄	28 d
	USGS-Columbia/ Huffman Lab (Boulder, CO)	DOC	CERC SOP B.5.21; EPA 415.2	Amber Glass	125 mL	40 mL	4°C / pH 2 H ₂ SO ₄	7 d / 28 d*

* holding time is 7 days for refrigerated/unacidified; 28 days if acidified

Table 10. Test acceptability requirements for acute and chronic water-only toxicity test with white sturgeon and rainbow trout (adapted from ASTM 2009a,b,c). Toxicity test should usually be considered unacceptable if one or more of the following occurred:

Item	Requirement
1	Fish used in the toxicity tests were obtained from spawning at fewer than 2 female and 2 male fish
2	Average control survival at the end of toxicity tests was: Acute tests: <90% Chronic Biphasic Stage 1 Exposure 1 (21-day starting with about 1 day post hatch (dph) fish): <80% Chronic Biphasic Stage 2 Exposure 2 (28-day starting with about 28 dph exogenous-feeding fish): <80% Chronic Continuous Test (56-day exposure starting with 1 dph fish): <64%
3	Average daily temperature should be $15 \pm 1^{\circ}\text{C}$ and instantaneous temperature should be within $\pm 3^{\circ}\text{C}$ of 15°C
4	Fish were not from the same source or fish were not acclimated for at least 2 days to test water and temperature
5	All test chambers (and compartments) were not identical
6	Treatments were not impartially assigned to test chamber locations
7	The test organisms were not impartially assigned to test chambers
8	Dilution water control was not included in a test
9	The test was started using organisms older than the ages specified in Tables 3 or 4
10	The test was ended before the duration specified in Table 4
11	Water quality characteristics and concentration of metals in test water were not measured as specified in Table 4
12	The time-weighted, average measured dissolved oxygen concentration at the end of a test for any test chamber as not between 80 and 100% of saturation
13	The measured concentration of test material in any treatment was less than 50% of the time-weighted average measured concentration for more than 10% of the duration of a chronic test
14	The measured concentration of test material in any treatment was more than 30% higher than the time-weighted average concentration for more than 5% of the duration of a chronic test