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1.0. Title Page

QUALITY ASSURANCE PROJECT PLAN
FOR THE HANFORD 100 AREA AQUATIC ASSESSMENT PROJECT

Study P00 -21-01 (Hanford); The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site Toxicity Test and Laboratory Avoidance Tests.

United States Geological Survey,
Columbia Environmental Research Center

Submitted To:

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2.0. Document Approval Form

Quality Assurance Project Plan Approval Form
USGS, Columbia Environmental Research Center

Project Title: The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site toxicity test and laboratory avoidance tests.

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Date

**NOTE: THE ORIGINAL VERSIONS OF ALL APPENDIXES TO THIS QAPP,
COMPLETE WITH APPROPRIATE SIGNATURES, ARE ON FILE AT THE US
GEOLOGICAL SURVEY, COLUMBIA ENVIRONMENTAL RESEARCH CENTER,
COLUMBIA, MO.**

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4.0 Introduction

This Quality Assurance Project Plan (QAPP) complies with the quality assurance policy established by the United States Environmental Protection Agency (USEPA) on monitoring and measurement projects. The USEPA policy stipulates that every monitoring and measurement project must have a written and approved QAPP. A QAPP is a written document, which presents in specific terms, the policies, organization (where applicable), objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities designed to achieve the data quality goals of a specific project (s) or continuing operation (s). The QAPP is prepared by the responsible Program Office, Regional Office, laboratory, contractor, grantee, or other organization.

This document describes the sixteen elements of a QAPP. These QAPP elements describe procedures which will be used to document and report precision, accuracy, and completeness of environmentally-related measurements. Environmentally-related measurements are defined as all field and laboratory investigations that generate data. These include (1) the measurement of chemical, physical, or biological parameters in the environment, (2) the determination of the presence or absence of pollutants in waste streams, (3) the assessment of health and ecological effects studies, (4) conduct of clinically and epidemiological investigations, (5) performance of engineering and process evaluations, (6) study of laboratory simulation of environmental events, and (7) study or measurement on pollutant transport and fate, including diffusion models.

5.0. Project Description

5.1. Background Information

The Environmental Contaminants Research Center (CERC) Research Study Plan 418, "The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA" was implemented in August of 1998. Study Plan 418 was amended in September of 1999 to include two additional studies described in Amendment I, "The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site Toxicity Test and Laboratory Avoidance Tests" of Appendix A. CERC Study Plan 418 describes a cooperative research project between the U.S. Geological Survey, Columbia Environmental Research Center, the U.S. Fish and Wildlife Service Upper Columbia River Basin Field Office, and the Hanford Natural Resource Trustees with participation by the U.S. Department of the Interior, U.S. Department of Commerce, U.S. Department of Energy, the Yakama Nation, the Confederated Tribes of the Umatilla Indian Reservation, the Nez Perce Tribe, and the States of Oregon and Washington. The goal of the project is to assess the injury to chinook salmon inhabiting the Hanford Reach of the Columbia River as a result of exposure to chromium.

5.1.1. Summary

The amendment to Study Plan 418 includes a project consisting of two separate research tasks as follows:

- Task I. On-site Early Life-stage Toxicity Test; determine the on-site toxicity of chromium-contaminated groundwater from the 100 Area to early life-stages of Columbia River chinook salmon. Specifically, the survival and hatching success of salmon eggs and the survival and subsequent growth of alevins exposed to dilutions of chromium-contaminated groundwater will be determined.
- Task II. Laboratory Avoidance Tests; determine the avoidance response of chinook salmon parr exposed to aqueous chromium concentrations representative of conditions in the Hanford Reach of the Columbia River.

The research activities within the project are described in the task-specific sections below.

TASK I: ON-SITE EARLY LIFE-STAGE TOXICITY TEST:

The objective of this task is to determine the on-site toxicity of chromium-contaminated groundwater to early life-stage chinook salmon from the Columbia River. Eyed eggs of chinook salmon will be exposed to chromium concentrations 11, 24, 54, 120, and 266 $\mu\text{g/L}$. The chromium concentrations will be obtained by diluting 100% groundwater with the appropriate volumes of ambient Columbia River water. The control treatment will be 100% ambient Columbia River water. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system (CERC SOP F20.E18, "Construction, Operation, Calibration, And Maintenance of the Proportional Diluter"). Temperature will be maintained at $5\pm 2^\circ\text{C}$ by chilling the exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

Eyed embryos of chinook salmon for the on-site toxicity test will be obtained from Priest Rapids, Washington. Eggs will be transported to the on-site testing facility and acclimated to exposure conditions before beginning the exposures. To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the median swim-up date.

During the exposure, egg mortality and hatching will be monitored and recorded daily. Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. The development of alevins will be monitored daily to document the sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright

plough, free swimming, and exogenous feeding following Dill (1977).

Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at median hatch, approximately at the midpoint between hatch and swim-up, swim-up, and termination (30 days post median swim-up). Samples will be frozen with liquid nitrogen, stored at -90°C and held for possible later analysis of chromium tissue residues or assessment of physiological impairment (DNA, lipid peroxidation). Three fish from each replicate will be collected at swim-up and termination, preserved in 10% neutral buffered formalin, and held for possible histological analyses. At the end of the exposure (30 days post median-hatch) all surviving alevins in each treatment will be measured for total length and weighed to determine growth. Fish will not be fed for 24 h prior to sampling.

TASK II: LABORATORY AVOIDANCE TESTS:

The goal of this task is to begin to assess the potential for chromium to influence fish distribution and habitat availability in the Hanford Reach of the Columbia River. The first step in this assessment is to determine whether chinook salmon parr exhibit an avoidance response to chromium under controlled laboratory conditions. Laboratory tests measure the response of salmon presented with a choice between a control condition and chromium-contaminated water; a potentially adverse stimulus. Precisely controlled conditions are necessary to ascribe the observed behavioral response to the presented stimulus. Water quality that closely simulates the ambient river conditions will be used to control variables such as hardness and pH that are known to affect the speciation, complexation, biological availability and toxicity of metals, such as chromium. Although test conditions will attempt to simulate many of the conditions experienced by fish in the field, the focus of laboratory tests will be to control the nature of the stimulus (aqueous chromium) and the conditions under which it is presented to the organism.

ANALYSIS OF WATER AND TISSUE (ALL TASKS):

To assess stability of Cr species (e.g., total Cr vs. Cr (VI) exposure concentrations), a 96-hour flow-through test will be conducted prior to conducting the definitive on-site Hanford toxicity test. The procedures for pretest will be identical to those used to conduct the definitive on-site test except that test duration will be shorter (96-h) and the exposure water will be sampled more frequently for analysis of total Cr and Cr (VI). The diluter system will be calibrated before beginning the pretest. The calibration is performed to ensure that appropriate amounts of the groundwater are delivered to the chemical mixing chambers of the diluter and that the diluter delivers the appropriate volumes of exposure water to each treatment. During the pretest eyed eggs of a surrogate salmonid species will be exposed to groundwater dilutions containing 11, 24, 54, 120 and 266 µg/L of Cr and a control treatment of Columbia River water. Four replicates of each Cr treatment will be tested.

During the definitive on-site test chromium exposure concentrations will be determined with the same methods used for analysis of chromium during the pretest. For analysis of total chromium, one hundred mL samples will be collected weekly from each treatment and filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO₃, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time samples are collected for total chromium analysis, one additional sample will be collected from the low, middle, and high chromium treatments,

extracted, and analyzed for speciation of chromium (SOP P.454).

For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

5.1.2. Overview

The Hanford Nuclear Reservation in south central Washington is a 900 square kilometer area claimed by the federal government in 1943 as a site for the production of plutonium (Geist 1995). The location was ideal because it was remote, sparsely populated, and most importantly, had a readily available supply of cold water from the Columbia River. Because of national security concerns, public access and river development projects were restricted until 1971 (Dauble and Watson, 1997). Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Ocorhynchus tshawytscha*). The 90 km section within the Hanford Reservation was not developed, and today, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem spawning occurs in the Columbia River (Dauble and Watson 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the mainstem Columbia River.

Large quantities of Columbia River water were used to cool nuclear reactors and cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within the pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the river and also entered ground water through leakage of pipes and seepage from retention areas. Today, groundwater at the Hanford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994). The hydraulic head of the ground water aquifers in the 100 Area (National Priority List Site) are higher in elevation than that of the Columbia River and results in discharge from the aquifer into the Columbia River through the river bottom, shoreline springs and seeps. The ground water is hydraulically connected to the river with peak aquifer discharges occurring during low river flows (fall and winter) and minimum aquifer discharges occurring during high river flows (spring and summer) (Geist et al. 1994).

The use of the Hanford Reach for fall chinook spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook runs have declined during the same time period. Spawning occurs in close proximity to the 100 Area where contaminated ground water is entering the River. Adult chinook spawn in variable water depths, water velocities, and substrate types (Swan et al. 1988). Spawning in the Hanford Reach begins in mid-October, peaks in mid-November, and ends in late November (Dauble and Watson 1997). Egg and fry development within the redds take place from mid-October to May during low river flows that result in peak aquifer discharges. Based on the mid-November peak redd abundance and ambient temperatures, eggs would become eyed in early December, hatch in late December, and alevins would emerge from the redds in late February. Upon emergence, fry move out of the main river channel into shallow, slow moving, near shore and backwater habitat (Dauble and Watson 1990, Dauble et al. 1989). Juveniles remain in the Hanford Reach from

February to mid-July feeding on macroinvertebrates (Becker 1973). Outmigrating begins in May and is usually completed by July at 5-7 months of age, 60-70mm in length, and 3-4 gm in weight (Olson and Foster 1956).

5.2. Current Site Information

Chromium is a contaminant of major concern and it is associated with 100 Area groundwater and seeps. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria (AWQC, 11 $\mu\text{g/L}$) for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA 1986) and the water quality standard for chronic exposure (10 $\mu\text{g/L}$) established by the State of Washington (WAC-173-201A-040). Previous studies on the effects of hexavalent chromium on chinook salmon (Olson and Foster 1956, Buhl and Hamilton 1991) did not investigate direct effects on fertilization, effects on health status of alevins, recovery of exposed alevins, or physiological impairment. Identification of effects and the range of concentrations at which those effects may be manifest is necessary to evaluate the potential for chromium discharge to cause injury to salmon populations in the Columbia River. In particular, additional information is needed to determine if the current standards protect survival and development early-life stages of chinook salmon.

5.3. Project Objectives

The objectives of this project are (1) to determine the on-site toxicity of chromium-contaminated groundwater from the 100 Area to early life-stages of Columbia River chinook salmon and (2) to determine the avoidance response of chinook salmon exposed to water qualities representative of conditions in the Columbia River.

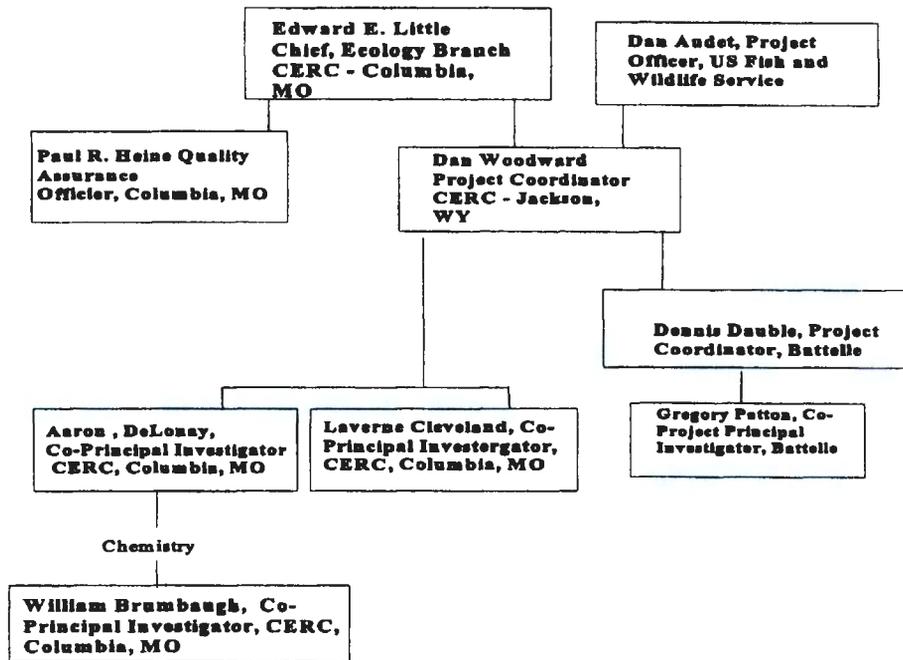
5.4. Project Schedule

PROJECT SCHEDULE		
ITEM	START	END
Study Design and Organization	Aug 1999	Oct 1999
<u>TASK I: On-site Early Life-stage Toxicity Test</u>		
perform tests	Oct 1999	Mar 2000
data analyses	Apr 2000	Jul 2000
submit draft report	Sept 2000	Oct 2000
submit final report	Oct 2000	
<u>TASK II: Laboratory Avoidance Tests</u>		
perform test	Feb 2000	Apr 2000
data analyses	May 2000	Aug 2000
submit draft report	Oct 2000	Nov 2000
submit final report	Dec 2000	

6.0. Project Organization and Responsibilities

The Project's organization and responsibilities are summarized in Figure 1. The qualifications of personnel to perform research and/or administrative and management activities associated with the Project are summarized in Appendix B in the form of curriculum vitae.

Figure 1. Distribution of overall responsibilities for project P00-21-01



6.1. Program Management

Dan Woodward of the CERC Field Research Station at Jackson, Wyoming will serve as Coordinator for the Project and will report to the CERC Ecology Branch Chief and the Project Officer for the U.S. Fish and Wildlife Service (Figure 1). Quality Assurance and Quality Control (QA/QC) functions for the Project will be the responsibility of Paul R. Heine, the CERC QA Officer. The QA Officer or his designee will conduct all QA/QC inspections and audits and will report to the Ecology Branch Chief. Gregory Patton, Pacific Northwest National Laboratories and Aaron DeLonay, CERC will serve as co-principal investigators for the on-site early life stage test under task I. Aaron DeLonay will report to CERC Ecology Branch Chief, Edward Little and Gregory Patton will report to Dennis Dauble, the project coordinator for Pacific Northwest National Laboratories. Dennis Dauble will report to Dan Audet. Aaron DeLonay and Laverne Cleveland will serve as co-principal investigators for the laboratory avoidance study under Task 2 of the Project and will report to the CERC Ecology Branch Chief Edward Little. William Brumbaugh will serve as Co-principal Investigator for Cr chemical analyses and will report to the respective co-principal investigator for task 2 studies (Figure2).

6.2. Quality Assurance Responsibility

Paul Heine will be responsible for conducting laboratory checks, identifying and controlling non-conformance, and initiating corrective actions whenever QA/QC limits are exceeded.

6.3. Laboratory Responsibilities

Under Task 1 of the Project Gregory Patton will be responsible for conducting the on-site early life-stage toxicity test and collecting the data on egg survival and hatch, and the growth and survival of alevins. Chemical analyses of chromium during the on-site test will be performed by Pacific Northwest National Laboratories (Figure 1). Under Task 2 of the Project, the laboratory avoidance tests will be performed by Aaron DeLonay and Laverne Cleveland. Bill Brumbaugh will be responsible for chemical analyses for each test under Task 2 (Figure 1).

7.0. Quality Assurance Objectives for Measurement Data

7.1. Precision, Accuracy, and Test Acceptability

7.1.1. Task 1- on-site early life stage toxicity test ; Task 2 - Laboratory avoidance tests.

Precision describes the degree to which data generated from replicated treatments differ. Accuracy is the difference between measured data values and the true data values. Precision and accuracy will be determined for tests under each Task by replicating treatments a minimum of four times. Treatment replication allows the estimation of standard deviation and coefficient of variation (CV) which are used as indicators of precision and accuracy. The acceptability of toxicity tests conducted under Task 1, and Task 2 will be assessed by their precision and responses observed for control treatments used in all tests.

7.1.2. Chemical Analyses

Precision and accuracy for chemical analyses performed for tests under Task 1 and Task 2 will be assessed by use of replicate analyses, certified reference materials, matrix spikes, procedural blanks, and control samples. Water (and tissue) samples will be analyzed for chromium according to CERC SOPs C5.212, P.454, C5.36, C5.94 and C5.163.

8.0. Analytical, Toxicity Testing, and Sampling Procedures

8.1. Analytical Procedures

8.1.1. Water Quality

Exposure water will be monitored for dissolved oxygen, pH, alkalinity, hardness, and conductivity following standard methods (APHA, 1975 and CERC SOPs B4.14, B4.15, B4.16, and B4.9).

8.1.1.1. Chromium Water Analyses

One hundred mL samples of exposure water will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO₃, and analyzed with ICP-MS according to CERC SOP C5.212. Water samples will be analyzed for chromium speciation according to CERC SOP P.454.

8.1.1.2. Chromium Tissue Analyses

Tissue samples will be collected from each chromium treatment, placed in polyethylene bags and frozen at -4 °C pending analysis. To analyze tissue samples for chromium the tissue samples will be lyophilized according to CERC SOP C5.36, acid digested with microwave heating (CERC SOP C5.94), and analyzed by either ICP-MS (CERC SOP C5.212) or graphite furnace atomic absorption spectrophotometry (CERC SOP C5.163).

8.1.1.3. Lipid Peroxidation

Samples for lipid peroxidation will be collected from the chromium treatments, frozen with liquid nitrogen, and stored at -90°C pending analysis according to Jackson SOP F20.25.

8.1.1.4. DNA Strand Breakage

Samples for DNA strand breakage will be collected from the chromium treatments, frozen with liquid nitrogen, and stored at -90°C pending analysis according to Jackson SOP F20.29.

8.1.1.5. Histology

Samples for histology will be collected from the chromium treatments and fixed in 10% neutral buffered formalin pending histological examinations. Gill tissue, liver tissue (free of the gall bladder), kidney, intestines, spleen, and skin will be examined for histological anomalies.

8.2. Toxicity Testing Procedures

The laboratories in which studies will be conducted will be restricted to authorized personnel. Protocol P00-21-01 used for conducting research described in Task I and II of the Project is included as Appendix C. A list of Standard Operating Procedures (SOPs) used for conducting the Protocol is attached to the Protocol (Appendix C). These SOPs are established methods which are followed during the conduct of research activities under the Protocol. The use of SOPs insures that (1) sound, scientifically acceptable technical guidelines will be used to perform all research activities described in the Protocol, (2) that work performed and data generated will be of the highest attainable quality, and (3) that all work performed will be thoroughly documented for purposes such as litigation. Examples of CERC SOPs are presented in Appendix D.

9.0. Sample Custody Procedures

Principal Investigators will be the custodians for all samples collected during the conduct of the Protocol and a strict chain of custody will be maintained. All samples collected for chromium water analysis, chromium tissue analysis, lipid peroxidation, DNA strand breakage, and histology will be labeled with a unique sample identification number and sealed with tamper resistant tape to discourage tampering. All samples will be stored in a secured coolers or freezers as appropriate, which will be continuously monitored for temperature. As necessary, samples will be shipped between the research facilities (PNNL and CERC) or to other analytical facilities in secured containers by overnight express courier service. A chain-of-custody form will accompany all samples which are shipped or otherwise transferred.

Data files and records will be maintained by the Principal Investigators during the conduct of studies and will be made available to the Project Coordinator upon request and at the completion of studies. Files and records will include all project documents, correspondences (telephone, mail, e-mail, and FAX), raw data records, laboratory logs, and project deliverables. Data from all Task II studies will be submitted to CERC Information Technology personnel for local archiving within four weeks after the studies are completed as outlined in CERC SOP B5.63. Study files will be permanently archived as outlined in CERC SOP B5.147. Data will be transmitted to the Hanford Project Officer in the form of a final report.

10.0. Calibration Procedures and Frequency

The instruments used for measuring chemical parameters such as chromium exposure water and tissue concentration, pH, dissolved oxygen, temperature, conductivity, alkalinity, and hardness are calibrated and standardized according to instrument manufacturers procedures. A list of these SOPs are attached to the Protocol (Appendix C). Records of calibration data, performance checks, and instrument service are maintained in permanent bound log notebooks or in computer files in a manner that the history of performance of the instruments may be easily reviewed. Standards such as NBS-certified thermometers for devices used to measure temperature, class-s weights for balances, and conductivity, hardness, and alkalinity standards and National Institute of Standards and Technology quality control solutions are used to insure that instruments function properly. Analytical reagents are labeled and dated upon receipt and are protected from deterioration. All instruments used to measure water quality are calibrated weekly, except dissolved oxygen and pH meters which are calibrated before being used.

11.0. Internal Quality Control Checks and Frequency, Performance and System Audits

The Quality Assurance Officer will conduct quality control audits at the end of all studies. All audit reports will be forwarded to the Principal Investigators and Project Coordinators for documentation of corrective action. The completed audit report will then be sent to the Quality Assurance officer for review. All studies will include control treatments. All study treatments will be replicated to provide a measure of precision as standard deviations and coefficients of variation. The acceptability of tests will be assessed by their precision and the response (e.g., survival, growth) of organisms exposed to the control treatments. For acceptable tests survival of organisms exposed to the control treatments should be $\geq 85\%$ and variation among replicated treatments should not exceed 20%. All deviations from the Protocol will be documented in the

form of a Protocol amendment which will undergo the same approval process as the Protocol. Results of chromium exposure water and tissue analyses will be accompanied by data validation information including duplicate results, standard reference material recoveries, spike results, procedural blank results, detection limits, calibration data, and summaries of deviations from the Protocol due to laboratory or sample problems.

12.0. Data Reduction, Validation, Assessment, and Reporting Procedures

Effects parameters will be calculated for each toxicity test. Effects parameters will include percentage survival, percentage inhibition of growth and mean weight loss or gain, mean behavioral responses, mean increases in lipid peroxidation, mean occurrences of DNA strand breakages, mean occurrences of histological anomalies, no observable effect concentrations (NOEC), lowest observable effect concentration. When appropriate each effect parameter will be accompanied by an indicator of variance associated with the effect (e.g., standard deviations, standard errors, coefficients of variations). Effects parameters will be determined by comparing the responses of organisms exposed to the chromium treatments to that of organisms exposed to the control treatments or comparison of responses among chromium treatments.

Response differences between treatments will be determined by use of appropriate statistical procedure such as analysis of variance (ANOVA) with means separation test (e.g., Fishers LSD, Duncan's, Tukeys). Statistical analyses will be performed with computer programs such as Statistical Analysis Systems (SAS) programs (SAS 1985).

All data will be evaluated by the Principal Investigator for each test, the Project Coordinator, and the Quality Assurance Officer. The results of tests will be reported to the Project Officer in the form of a final report.

13.0. Preventive Maintenance Procedures and Schedules

Records are kept on all analytical equipment. Calibration checks are used to determine when probes, diffusion membranes, batteries, and buffer solutions need to be changed. Reference standards are replaced on their expiration dates. Refer to the list of SOPs in Appendix C for additional information.

14.0. Corrective Actions

Corrective actions will be implemented whenever research equipment is not in compliance. Details on corrective actions are described for specific research equipment and research activities in CERC SOPs. Corrective actions are based on data acceptability limits (e.g., analytical standards values not within specified range) or identification of equipment defects. Measures will be implemented to correct defects underlying non compliance, document the results of corrective actions, and monitor activities or equipment until compliance is established. In cases of non compliance, corrective actions will be immediate and most often will be implemented by the analyst. Corrective actions may also result from QA Audits. Most often issues requiring corrective actions are related to equipment malfunction or failure or deviations from QA objectives.

15.0. Quality Assurance Reports to Management

Written QA reports will be initiated at the request of the Project Coordinator or Project Officer or as a result of a QA audit. The QA officer will be responsible for all QA reports. The reports will include information such as a description of discrepancies or problems, descriptions of variances from QA objectives, implications of the variances, discrepancies, or problems, required corrective actions, and a schedule for meeting corrective actions.

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17.0. Appendix A. Columbia Environmental Research Center Research Study Plan 418

ENVIRONMENTAL AND CONTAMINANTS RESEARCH
CENTER RESEARCH STUDY PLAN 418

Study Plan Title: The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA.

BRD SIS Project Number:

Date Prepared: 08/17/98

Date Revised: 09/09/99

BRD Center: Columbia Environmental Research Center

Facility Contact: Wilbur L. Mauck

Project Contact: Daniel Woodward

Date Initiated: 1998

I. Rational and Justification: Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Ocorhynchus tshawytscha*). An area that did not experience development is a 90 km section within the Hanford Nuclear Reservation which was claimed by the federal government in 1943 as a site for the production of plutonium. Currently, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem chinook salmon spawning occurs in the Columbia River (Dauble and Watson 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the Columbia River. The use of the Hanford Reach for fall chinook spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook runs have declined during the same time period.

Plutonium production within the Hanford Reservation required the use of large quantities of Columbia River water to cool nuclear reactors. The cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within cooling system pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the Columbia River and also entered ground water through leakage of pipes and seepage from retention areas. Currently, groundwater at the Hanford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994).

The Hanford Reach of the Columbia River is critically important as spawning habitat for the chinook salmon and it is essential to determine if current water quality standards protect

chinook salmon (Geist 1997). Chromium is one contaminant of major concern and it is associated with the 100 Area (National Priority List Site) groundwater and seeps. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria of (AWQC) of 11 $\mu\text{g/L}$ for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA 1986) and the State of Washington (WAC-173-201A-040). In particular, additional information is needed to determine if the current standards protect survival and development early-life stages of chinook salmon. Previous studies on the effects of hexavalent chromium on chinook salmon (Olson and Foster 1956, Buhl and Hamilton 1991) did not investigate direct effects on fertilization, effects on health status of alevins, recovery of exposed alevins, or physiological impairment.

II. Objectives (what): The objectives of this study are to assess the effects of chromium on chinook salmon under exposure conditions similar to those of the Hanford Reach of the Columbia River.

III. Listing of Studies:

A. **Study 1: Fertilization: The potential for chromium to adversely affect gametes and their fertilization in chinook salmon.**

1. Principal Investigator(s): Daniel F. Woodward and Aida Farag
2. Specific Objectives: **To determine the toxicity of chromium to chinook salmon ovum, sperm, and the fertilization process.**
3. Experimental Design or Methodological Approach: Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as CaCO_3 ; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 $\mu\text{g/L}$. This range of concentrations are above and below the chronic

AWQC for chromium, 11 $\mu\text{g/L}$ (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996).

Gametes will be obtained from reproductively mature chinook salmon from the McNenny State Fish Hatchery, Spearfish, South Dakota. The salmon from which the gametes are obtained will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

Gametes will be taken from chinook salmon brood stock between October and November of 1998. This is the normal time for gametogenesis in fall adult chinook salmon and the stock will be checked weekly for ovum and sperm formation. We will use a pooled source of eggs and sperm from a minimum of three females and three males to perform three tests: 1) toxicity of chromium to the ovum, the ovum survival test; 2) toxicity of chromium to sperm, the sperm survival test; and 3) toxicity of chromium to fertilization, the fertilization test.

A physiological saline (PS) solution will be used in the ovum and fertilization test; and a physiological saline solution with sperm extender (PS/SE) will be used in the sperm test (Billard and Roubaud 1985). The physiological saline solution will consist of a standard 1% NaCl solution buffered to pH 9.0; the PS/SE will be the same solution with KCl added (30 mM) to prevent the spermatozoa from becoming motile. Ova and sperm can survive for several hours in these solutions. In all three tests, there will be six treatment concentrations of chromium: 0, 5, 11, 24, 54, and 120 $\mu\text{g/L}$. The six chromium treatments will be incorporated into the PS or the PS/SE so that when diluted with the appropriate amount of sperm or ovum the desired concentration of chromium is achieved. Each treatment will be replicated four times for a total of 24 treatments.

Ovum survival test. Ova will be divided into 24 treatment lots of 150-200 eggs, each and mixed with 10 ml of PS containing the appropriate chromium concentration. After 15 min, the liquid will be removed from each treatment and replaced with 10ml fresh uncontaminated PS solution followed by insemination with 1ml of intact sperm. This will be a 15 min ovum exposure.

Sperm survival test. Sperm will be diluted with PS/SE containing the appropriate chromium concentration (1ml sperm:10ml PS/SE) to obtain 24 treatment lots. The sperm, PS/SE, and chromium will be mixed, and left standing for 15 min. Sperm will be separated from the PS/SE exposure treatments by centrifuging for 10 min at 1800g, followed by replacement of 10ml fresh uncontaminated PS/SE. The exposed sperm will be used to inseminate 24 lots of about 150-200 ova, each previously diluted in 10ml of PS. This will be a 15 min sperm exposure.

Fertilization test. Ova (150-200), sperm (1ml), and 10ml of PS containing the appropriate chromium concentrations will be mixed together to achieve the 24 treatment lots. This will be a 65 min exposure of egg and sperm during fertilization and water

hardening. In all three tests, ova and sperm will be mixed for 5 min followed by rinsing and water hardening in Hanford experimental water according to standard procedures (Piper et al. 1982). Water hardening will last for one hour and is the process by which water is absorbed into the eggs and fills the perivitelline space between the shell and yoke. The eggs become turgid during this process and additional water exchange is minimal during further development. In the fertilization test, exposure to chromium will continue through water hardening. After water hardening, eggs will be rinsed and transferred into incubators. Eggs from all three tests will be incubated in McNenny hatchery water (temperature, 11°C; hardness, 360 mg/L as CaCO₃; alkalinity, 210 mg/L; and pH, 7.6) for 10 days. The eggs will be cleared in 10% acetic acid solution for 2 min and percent fertilization will be determined. The embryo of fertilized eggs will turn an opaque white and become visible through the translucent chorion. At 10 days the embryo will have a definite optic lobe developed with an elongated somite and will be easily distinguished from an unfertilized germinal disk.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO₃, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

4. **Listing of Critical Data:** **Objective: To determine the toxicity of chromium to chinook salmon ovum, sperm, and the fertilization process.** .
Data gathered from this objective will include chinook salmon ova survival, sperm survival, and percent fertilization at chromium concentrations representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn.
5. **Statistical Treatment:** Percent data for fertilization, ova survival, and sperm survival will be arc sine transformed and analyzed with Analysis of Variance (ANOVA) followed by Tukey means comparisons test. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
6. **Acceptance or Rejection Criteria for Results:** Acceptance or rejection criteria for test results are outlined in ECRC Standard Operating Procedures and standardized methods for specific studies. Appropriate Standard Operating Procedures and standardized methods used for these tests are cited in ECRC Protocol P99-10-01 (Attachment I).
7. **Special Safety Requirements:** Safety requirements for all research activities at the ECRC

are given in the ECRC Safety Plan.

8. **Endpoint of Study, based on Accomplishments:** This study will provide an assessment of the effects of chromium on the reproductive process, growth, survival, behavior, health status, and histopathology of chinook salmon. This study will end with the analysis and interpretation of the data gathered and the submission of a final report to the funding agency, the U.S. Fish and Wildlife Service. Data from this research will also be published in peer-reviewed journals.
9. **Schedule of Study and the Outputs Expected:**

STUDY SCHEDULE AND OUTPUTS		
ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
perform tests	Nov 98	Dec 98
data analyses	Jan 99	Mar 99
Submit draft report	Mar 00	Jun 00
Submit final report		90 days after receipt of comments

10. **Place Where Data will be Stored and Archived:** File folders of original data will be maintained at the ECRC Field Research Station, Jackson, WY and the ECRC, Columbia, MO according to standard operating procedures. Files from this study will be permanently archived by the ECRC QA/QC Officer as outlined in ECRC SOP B5.147.
11. **Relationship to Cooperator Needs:** The data gathered from this study will (1) provide an assessment of injury to chinook salmon exposed to chromium in the Hanford area of the Columbia River Basin, (2) will be useful in recovering damages for lost resources and in evaluating remedial options, including immobilization, treatment, and the no-action alternative; and (3) will be useful to Trustees, including the U.S. Fish and Wildlife Service, Hanford Natural Resource Trustees, the U.S. Department of the Interior, and the States of Oregon and Washington in efforts to manage the Columbia River salmon population.
12. **Literature Cited:** See literature cited section of Study 3 below.

13. Signatures:

Prepared by: _____ Date: _____
Laverne Cleveland
Principal Investigator

Approved by: _____ Date: _____
Edward E. little
Branch Chief

Approved by: _____ Date: _____
Aida Farag
Principal Investigator

Approved by: _____ Date: _____
Daniel F. Woodward
Principal Investigator

Approved by: _____ Date: _____
I. Eugene Greer
Animal Care and Use Committee Chair

Approved by: _____ Date: _____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date: _____
Wilbur L. Mauck
Center Director

B. **Study 2: Early Life Stage; Determine the effects of chromium on the early development of chinook salmon.**

1. Principal Investigator(s): Aaron DeLonay and Laverne Cleveland
2. Specific Objectives: (1) **To determine the effects of chromium on chinook salmon egg survival, egg hatching and alevin survival, growth, and behavioral development;** (2) **To evaluate the bioconcentration of chromium by chinook salmon and determine effects chromium exposure on DNA strand breakage, and lipid peroxidation (see Study 3 for further details of physiology measurements).**
3. Experimental Design or Methodological Approach: Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as CaCO₃; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 µg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 µg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Geist 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

Eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

Eyed eggs of chinook salmon will be exposed to 5, 11, 24, 54, and 120 µg/L of chromium and a control treatment with no chromium added. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system (ECRC SOP F20.E18, "Construction, Operation, Calibration, And Maintenance of the Proportional

Diluter"). Temperature will be maintained at $5\pm 2^\circ\text{C}$ by chilling the exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the medium swim-up date.

During the exposure, egg mortality and hatching will be monitored and recorded daily. Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. The development of alevins will be monitored daily to document the sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright plough, free swimming, and exogenous feeding following Dill (1977). The tanks will be video taped weekly to develop an accurate count of these developmental patterns among the test populations. Quantitative measures of the form and frequency of movements will be made during the alevin/free swimming transition.

Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at 0, 7, 14, and 28 days post median hatch date and analyzed for tissue residues of chromium, DNA strand breakage, and lipid peroxidation (see Task 3 for further details of physiology measurements). Two fish from each replicate will be collected on days 0 and 24 for histological analyses. At the end of the exposure (30 days post median-hatch) all surviving alevins in each treatment will be measured for total length and weighed to determine growth.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3 , and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

4. Listing of Critical Data: **Objective 1: To determine the effects of chromium on**

chinook salmon egg survival, egg hatching and alevin survival, growth, and behavioral development.

Data gathered from this objective will include chinook salmon egg survival, egg hatching, and alevin survival, growth, and behavioral development at chromium concentrations representative of concentrations in locations where salmon spawn.

(2) To evaluate the bioconcentration of chromium by chinook salmon and determine effects chromium exposure on DNA strand breakage, and lipid peroxidation (see Study 3 for further details of physiology measurements).

Data gathered from this objective will include chinook salmon chromium tissue residues, DNA strand breakage, lipid peroxidation and physiological development at chromium concentrations representative of concentrations in locations where salmon spawn.

5. Statistical Treatment: Data will be analyzed by use of Analysis of Variance (ANOVA) followed by Tukey means comparisons test. Percent data will be arc sine transformed before analysis. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
6. Acceptance or Rejection Criteria for Results: Acceptance or rejection criteria for test results are outlined in ECRC Standard Operating Procedures and standardized methods for specific studies. Appropriate Standard Operating Procedures and standardized methods used for this test are cited in ECRC Protocol P99-10-01 (Attachment I).
7. Special Safety Requirements: Safety requirements for all research activities at the ECRC are given in the ECRC Safety Plan.
8. Endpoint of Study, based on Accomplishments: This study will provide an assessment of the effects of chromium on the reproductive process, growth, survival, behavior, health status, and histopathology of chinook salmon. This study will end with the analysis and interpretation of the data gathered and the submission of a final report to the funding agency, the U.S. Fish and Wildlife Service. Data from this research will also be published in peer-reviewed journals.
9. Schedule of Study and the Outputs Expected:

STUDY SCHEDULE AND OUTPUTS		
ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
perform test	Nov 98	Mar 99
tissue analyses	Mar 99	Sep 99
data analyses	Oct 99	Dec 99
Submit draft report	Mar 00	Jun 00
Submit final report		90 days after receipt of comments

10. Place Where Data will be Stored and Archived: File folders of original data will be

maintained at the ECRC Field Research Station, Jackson, WY and the ECRC, Columbia, MO according to standard operating procedures. Files from this study will be permanently archived by the ECRC QA/QC Officer as outlined in ECRC SOP B5.147.

11. Relationship to Cooperator Needs: The data gathered from this study will (1) provide an assessment of injury to chinook salmon exposed to chromium in the Hanford area of the Columbia River Basin, (2) will be useful in recovering damages for lost resources and in evaluating remedial options, including immobilization, treatment, and the no-action alternative; and (3) will be useful to Trustees, including the U.S. Fish and Wildlife Service, Hanford Natural Resource Trustees, the U.S. Department of the Interior, and the States of Oregon and Washington in efforts to manage the Columbia River salmon population.
12. Literature Cited: See "Literature Cited" section of study 3 below.

13. Signatures:

Prepared by: _____ Date: _____
Laverne Cleveland
Principal Investigator

Approved by: _____ Date: _____
Edward E. Little
Branch Chief

Approved by: _____ Date: _____
Aaron Delonay
Principal Investigator

Approved by: _____ Date: _____
I. Eugene Greer
Animal Care and Use Committee Chair

Approved by: _____ Date: _____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date: _____
Wilbur L. Mauck
Center Director

- C. **Study 3: Fish Health; Determine degree of fish health impairment of chinook salmon exposed to chromium.**
1. **Principal Investigator(s)**: Daniel F. Woodward and Aida Farag
 2. **Specific Objectives**: (1) **Gather data on chromium-induced physiological responses to aid in the interpretation of growth and survival responses documented during the early life-stage test (Study 2 above).**
(2) **Interpret the physiological effects of chromium on fish and define parameters that can be measured on fish samples collected from the Hanford Reach.**
 3. **Experimental Design or Methodological Approach**: Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as CaCO₃; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 µg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 µg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Geist 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

Eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

The goals of this experiment are two-fold. First, data gathered from this experiment will further explain toxicological responses on growth and survival documented during the early life-stage experiment. Because fry at the end of the early life-stage experiment are small, it will be difficult to interpret the results in terms of

individual tissue responses. And, therefore, explain the mechanistic processes involved during chromium action on fish. Second, this experiment will provide useful information to interpret effects of chromium on fish in the Hanford Reach. Health parameters used in the laboratory can also be performed on fish collected in the field.

Measurements will be performed to assess physiological impairment caused by chromium. For example, researchers have documented that chromium causes lipid peroxidation (Susa et al. 1996). Lipid peroxidation results in damage to polyunsaturated fatty acids located in the cell membrane. This damage can decrease fluidity, increase leakiness, and inactivate membrane-bound enzymes. An ultimate result may be cell death and tissue damage (Halliwell and Gutteridge 1985, Wills 1985). Chromium can form intermediates that react with DNA (Outridge and Scheuhammer 1993). These reactions have been associated with DNA damage measured in the form of DNA strand breakage (Aiyar et al. 1990). Therefore, lipid peroxidation and DNA strand breakage will be measured, in addition to histology and tissue metal accumulation, to document physiological impairment during this study.

Eyed eggs will be maintained in a Heath^R incubator at a temperature of $10 \pm 2^\circ\text{C}$ and hardness of approximately 150 mg CaCO_3/L . Mortalities will be documented and removed daily. At hatch, the fish will be moved to flow-through culture tanks with a flow of 4 L/min. The fish will be fed at least a 5% wet weight ration of a commercial biodiet daily. The daily food ration will be split between two feedings.

The experimental phase will begin during the parr stage of fish by randomly distributing 35 fish in each of 12 test chambers receiving experimental water with a flow-through proportional diluter system (Jackson SOP: F.P19). The circular chambers will have a 20-L capacity with dimensions of 43.2 cm X 35.6 cm and a volume of 20,510 cubic cm. The fish will be allowed to acclimate in the experimental chambers for at least five days before the start of the experiment. Thus, the experiment will be conducted for a period of 100 days beginning with parr fish. Eyed embryos, larvae, and parr will be handled so as to minimize stress in accordance with the ECRC-Columbia Animal Welfare Plan and the Region 6 U.S. Fish and Wildlife Service, Fish Health Policy.

Chromium in stock solutions will be delivered to eight test chambers via automatic pipettes (Micromedic Systems AP, Model #25000FW). Two test concentrations of 11 and 24 $\mu\text{g}/\text{L}$ chromium (referred to from this point as 11 and 24 will be maintained in each of four replicate chambers. Four chambers without chromium added will be used as controls. Thus, a total of 12 experimental (four control, four with 11 $\mu\text{g}/\text{L}$ Chromium, and four with 24 $\mu\text{g}/\text{L}$) units will be maintained. Each chamber will receive 8 L/hr for 10 volume additions per day. Experimental units will be checked daily for mortality and observations on behavior.

At Day 60 and at the termination of the experiment, samples will be collected for fish health measurements. An external necropsy assessment will be made on all sacrificed fish (UW SOP P.1) and lengths and weights will be recorded. One whole fish will be collected from each replicate chamber for measurements of tissue metal accumulation. DNA strand breakage, lipid peroxidation, histological anomalies, and tissue metal accumulation will be evaluated in 2 to 4 fish from each replicate. Gill

lamellae, liver (free of the gall bladder), kidney, and intestine will be removed immediately from the 10 individual fish. Samples for histology will be collected from 2 fish from each replicated chamber and fixed in 10% neutral buffered formalin. It should be noted that spleen and skin samples will also be collected for histological examinations. Samples for DNA strand breakage, lipid peroxidation, and tissue metals will be collected from four fish from each replicate, frozen with liquid nitrogen, and stored at -90°C. At a later date, these samples will be ground with liquid nitrogen and composited by tissue to result in one sample from each replicate chamber. Aliquots of these composites will be measured for DNA strand breakage (Jackson SOP F20.29), lipid peroxidation (Jackson SOP F20.25), and tissue metals.

Samples will be collected from the remaining two fish for additional measurements of DNA strand breakage. For example, both the anterior and posterior portions of the kidney will be sampled to distinguish between effects related to immune functions of the kidney (i.e. anterior section) to effects on the excretory processes (i.e. posterior portion). This information can be used to explain the mechanisms of observed toxicity. These data are also necessary to make comparisons between data gathered from samples of whole, ground tissue and those from specific locations within a tissue. Whole tissue samples would be less labor intensive to collect in field situations. However, it must be documented that this method is sufficiently sensitive to correspond with other toxicological effects. Fish will not be fed for 24 h prior to sampling.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO₃, and analyzed with ICP-MS (See: ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

4. **Listing of Critical Data: Objective 1: Gather data on chromium-induced physiological responses to aid in the interpretation of growth and survival responses documented during the early life-stage test (Study 2 above).**
Objective 2: To compare laboratory-derived chromium-induced effects on health parameters of chinook salmon with health parameters measured on field-collected salmon from the Hanford Reach of the Columbia river.
Data gathered from these two objectives will include chinook salmon survival, behavior, lipid peroxidation, DNA strand breakage, histology and tissue metal accumulation at chromium concentrations representative of concentrations in locations where salmon spawn.

5. Statistical Treatment: Data will be analyzed by use of Analysis of Variance (ANOVA) followed by Tukey means comparisons test. Percent data will be arc sine transformed before analysis. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
6. Acceptance or Rejection Criteria for Results: Acceptance or rejection criteria for test results are outlined in ECRC Standard Operating Procedures and standardized methods for specific studies. Appropriate Standard Operating Procedures and standardized methods used for this test are cited in ECRC Protocol P99-10-01 (Attachment I).
7. Special Safety Requirements: Safety requirements for all research activities at the ECRC are given in the ECRC Safety Plan.
8. Endpoint of Study, based on Accomplishments: This study will provide an assessment of the effects of chromium on the reproductive process, growth, survival, behavior, health status, and histopathology of chinook salmon. This study will end with the analysis and interpretation of the data gathered and the submission of a final report to the funding agency, the U.S. Fish and Wildlife Service. Data from this research will also be published in peer-reviewed journals.
9. Schedule of Study and the Outputs Expected:

STUDY SCHEDULE AND OUTPUTS		
ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
perform tests	Mar 99	Jun 99
tissue analyses	Jun 99	Dec 99
data analyses	Jan 00	Mar 00
Submit draft report	Mar 00	Jun 00
Submit final report		90 days after receipt of comments

10. Place Where Data will be Stored and Archived: File folders of original data will be maintained at the ECRC Field Research Station, Jackson, WY and the ECRC, Columbia, MO according to standard operating procedures. Files from this study will be permanently archived by the ECRC QA/QC Officer as outlined in ECRC SOP B5.147.
11. Relationship to Cooperator Needs: The data gathered from this study will (1) provide an assessment of injury to chinook salmon exposed to chromium in the Hanford area of the Columbia River Basin, (2) will be useful in recovering damages for lost resources and in evaluating remedial options, including immobilization, treatment, and the no-action alternative; and (3) will be useful to Trustees, including the U.S. Fish and Wildlife Service, Hanford Natural Resource Trustees, the U.S. Department of the Interior, and the States of Oregon and Washington in efforts to manage the Columbia River salmon population.

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Wilbur L. Mauck
Center Director

AMENDMENT I TO CERC RESEARCH STUDY PLAN 418

CERC Research Study Plan 418 is amended to include an additional project as described below.

Study Plan Title: The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site Toxicity Test and Laboratory Avoidance Tests.

USGS SIS Project Number: 418

Date Amendment Prepared: 09/09/99

Date Revised: 12/20/99

USGS Center: Columbia Environmental Research Center

Facility Contact: Wilbur L. Mauck

Project Contact: Daniel Woodward

Date Initiated: 1999

I. Rational and Justification: Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Ocorhynchus tshawytscha*). An area that did not experience development is section within the Hanford Nuclear Reservation which was claimed by the federal government in 1943 as a site for the production of plutonium. Currently, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem chinook salmon spawning occurs in the Columbia River (Dauble and Watson 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the Columbia River. The use of the Hanford Reach for fall chinook spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook runs have declined during the same time period.

Plutonium production within the Hanford Reservation required the use of large quantities of Columbia River water to cool nuclear reactors. The cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within cooling system pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the Columbia River and also entered ground water through leakage of pipes and seepage from retention areas. Currently, groundwater at the Hanford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994).

The Hanford Reach of the Columbia River is critically important as spawning habitat for the chinook salmon and it is essential to determine if current water quality standards protect chinook salmon (Geist 1997). Chromium is one contaminant of major concern and it is associated with the 100 Area (National Priority List Site) groundwater and seeps. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria (AWQC, 11 $\mu\text{g/L}$) for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA 1986) and the water quality standard for chronic exposure (10 $\mu\text{g/L}$) established by the State of Washington (WAC-173-201A-040). Previous studies on the effects of hexavalent chromium on chinook salmon (Olson and Foster 1956, Buhl and Hamilton 1991) did not investigate direct effects on fertilization, effects on health status of alevins, recovery of exposed alevins, or physiological impairment. Identification of effects and the range of concentrations at which those effects may be manifest is necessary to evaluate the potential for chromium discharge to cause injury to salmon populations in the Columbia River. In particular, additional information is needed to determine if the current standards protect survival and development early-life stages of chinook salmon.

II. Objectives (what): The objectives of this project are (1) to determine the on-site toxicity of chromium-contaminated groundwater from the 100 Area to early life-stages of Columbia River chinook salmon and (2) to determine the avoidance response of chinook salmon parr exposed to aqueous chromium concentrations representative of conditions in the Hanford Reach of the Columbia River.

III. Listing of Studies:

A. Study 1: Task I: On-site Early Life-stage Toxicity Test:

1. Principal Investigator(s): Aaron DeLonay, CERC; Gregory Patton, Pacific Northwest National Laboratories
2. Specific Objectives: (1) to determine the on-site toxicity of chromium-contaminated groundwater from the 100 Area to early life-stages of Columbia River chinook salmon. Specifically, the survival and hatching success of salmon eggs and the survival and subsequent growth of alevins exposed to dilutions of chromium-contaminated groundwater will be determined.
3. Experimental Design or Methodological Approach:
Task I. The purpose of this task is to determine the on-site toxicity of chromium-contaminated groundwater to early life-stage chinook salmon from the Columbia River. Eyed eggs of chinook salmon will be exposed to chromium concentrations 11, 24, 54, 120, and 266 $\mu\text{g/L}$. The chromium concentrations will be obtained by diluting 100% groundwater with the appropriate volumes of ambient Columbia River water. The control treatment will be 100% ambient Columbia River water. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system (CERC SOP F20.E18, "Construction, Operation, Calibration, And Maintenance of the Proportional Diluter"). Temperature will be maintained at 5 ± 2 °C by chilling the

exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the median swim-up date. During the exposure, egg mortality and hatching will be monitored and recorded daily.

Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. Visual observations will be conducted daily to monitor the development of alevins. The sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright plough, free swimming, and exogenous feeding will be documented following Dill (1977).

The following samples will be collected during the experiment for measurements of fish health. This assessment of physiological impairment will be performed if funds become available. Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at median hatch, approximately at the midpoint between hatch and swim-up, swim-up, and termination (30 days median hatch date). Samples will be frozen with liquid nitrogen, and stored at -90°C for later measurements of DNA strand breakage, lipid peroxidation, and tissue chromium measurements. Three fish from each replicate will be collected at swim-up and termination, preserved in 10% neutral buffered formalin, and held for histological analyses. The number of samples collected for fish health are listed in Table 1. Poor hatching success or high mortality may require intermediate sampling dates to be eliminated or the number of samples to be reduced. Changes in sampling frequency or number will be made in consultation with project coordinators. At the end of the exposure (30 days post median-hatch) an external necropsy assessment will be made on all surviving alevins, and lengths and weights will be recorded. Fish will not be fed for 24 h prior to sampling.

Table 1. Total number of whole-fish samples collected during Task I on-site ELS test.

TASK I - Onsite Early Life-stage Toxicity Test							
SAMPLE DAY	CONCENTRATION ($\mu\text{g/L}$)						TOTAL
Median Hatch	0	11	24	54	120	266	
Tissue metal, DNA, LP ¹	4	4	4	4	4	4	24
Mid-point Between Hatch and Swim-up							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Swim-up							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Histology	12	12	12	12	12	12	72
Termination							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Histology	12	12	12	12	12	12	72

¹LP = Lipid peroxidation

Total tissue metal, DNA, and LP for Task 1 = 96

Total histology for Task 1 = 108

B. Study 2. TASK II: Laboratory Avoidance Tests:

1. Principal Investigator(s): Aaron DeLonay, CERC; Laverne Cleveland (CERC), William Brumbaugh (CERC)
2. Specific Objectives: to determine the avoidance response of chinook salmon parr exposed to aqueous chromium concentrations representative of conditions in the Hanford Reach of the Columbia River.
3. Experimental Design or Methodological Approach:
 Task II. The goal of this task is to begin to assess the potential for chromium to influence fish distribution and habitat availability in the Hanford Reach of the Columbia River. The first step in this assessment is to determine whether chinook salmon parr exhibit an avoidance response to chromium under controlled laboratory conditions. Laboratory tests measure the response of salmon presented with a choice between a control condition and chromium-contaminated water; a potentially adverse stimulus. Precisely controlled conditions are necessary to ascribe the observed behavioral response to the presented stimulus. Water quality that closely simulates the ambient river conditions will be used to control variables such as hardness and pH that are known to affect the speciation,

complexation, biological availability and toxicity of metals, such as chromium. Although test conditions will attempt to simulate many of the conditions experienced by fish in the field, the focus of laboratory tests will be to control the nature of the stimulus (aqueous chromium) and the conditions under which it is presented to the organism.

Two avoidance experiments will be conducted. The first will determine the avoidance response of chinook salmon to chromium concentrations ranging from 0 to 120 $\mu\text{g/L}$ in reconstituted, experimental water (80 mg/L hardness as CaCO_3 , $10 \pm 2^\circ \text{C}$). Selected concentrations were based on the chronic EPA ambient water quality criteria for chromium (11 $\mu\text{g/L}$), concentrations that are expected to elicit an avoidance response based on a survey of the literature, and the range of concentrations expected in the Hanford Reach of the Columbia River.

The second series of avoidance tests is designed to examine whether the water quality characteristics associated with a groundwater source will alter the response of chinook salmon to chromium. Chromium is associated with seeps and areas of upwelling, contaminated groundwater that exist along the river's edge, and in the river bed. Water quality characteristics associated with upwelling groundwater may alter the avoidance response by either changing the perception or toxicity of the stimulus (chromium), or by presenting water quality conditions (hardness, alkalinity, pH, etc.) which may be preferred over the control condition. The second experiment will evaluate the response of chinook salmon to a simulated groundwater (200 mg/L hardness as CaCO_3) with and without the addition of aqueous chromium. The test combinations for each experiment are illustrated in Table 2.

Juvenile chinook salmon (0.25 to 2.0 grams) will be acclimated to, and maintained in experimental water (80 mg/L hardness) at the test temperature ($10 \pm 2^\circ \text{C}$) for a minimum of two weeks prior to the start of avoidance experiments. Fish will not be fed 24 hours prior to testing. Avoidance tests will be conducted using a counter-current apparatus in accordance with CERC SOP B5.232. A Control (experimental water without chromium) and a test solution (experimental water with chromium) flow in from opposite ends of the apparatus, and exit from six drains at the center. This apparatus produces a steep, central gradient between the control and test treatment. Prior to the start of the experiments the apparatus will be calibrated and the steepness of the gradient verified using fluorescein dye.

Individual fish will be placed into each of three avoidance apparatus. After sufficient acclimation (indicated by exploratory behavior) the test will begin. Acclimation times are species and life-stage dependant and usually range from 20 to 40 minutes. The test period will be 40 minutes in duration. The behavioral response to the gradient will be recorded on video tape and analyzed in ten minute intervals as the proportion of time spent in the test solution versus the time spent in the control solution. A series of trials will be conducted using three apparatus, each presenting test organisms with the control and one of the test conditions. This series of treatment combinations will be replicated a

minimum of eight times. The test condition delivered to each test apparatus, and the end of the apparatus receiving the test condition will be randomized and alternated between trials. The control combination is a test in which experimental water without contaminants flows into both ends of the apparatus.

Tests will be discarded if there is a disturbance to the avoidance apparatus; inconsistent water chemistry, temperature, or quality; or disease, aggression, or abnormal behavior. Tests will also be discarded if test organisms do not cross the gradient a minimum of three times during the test period. The apparatus will be enclosed in a structure to shield against external movement or sound. Water quality characteristics (pH, alkalinity, hardness, and conductivity) of the reconstituted Columbia River water and Hanford groundwater will be sampled daily.

Table 2. Experimental matrix of treatment combinations used for Task II avoidance tests.

Experiment I	
Control Condition	Test Conditions
Experimental Water ¹ + 0 μg/L Cr	Experimental Water + 0 μg/L Cr
	Experimental Water + 11 μg/L Cr
	Experimental Water + 27 μg/L Cr
	Experimental Water + 54 μg/L Cr
	Experimental Water + 120 μg/L Cr
Experiment II	
Control Condition	Test Conditions
Experimental Water + 0 μg/L Cr	Simulated Groundwater ² + 0 μg/L Cr
	Simulated Groundwater + 11 μg/L Cr
	Simulated Groundwater + 27 μg/L Cr
	Simulated Groundwater + 54 μg/L Cr
	Simulated Groundwater + 120 μg/L Cr

¹ Experimental Water (80 mg/L hardness as CaCO₃)

² Simulated Groundwater (200 mg/L hardness as CaCO₃)

ANALYSIS OF WATER (ALL TASKS):

Prior to conducting the definitive on-site Hanford toxicity test (Task I), a 96-hour flow-through test will be conducted to assess stability of chromium species (e.g., total Cr vs. Cr (VI) exposure concentrations). The procedures for pretest will be identical to those used to conduct the definitive on-site test except that test duration will be shorter (96-h)

and the exposure water will be sampled more frequently for analysis of total Cr and Cr (VI). The diluter system will be calibrated before beginning the pretest. The calibration is performed to ensure that appropriate amounts of the groundwater are delivered to the chemical mixing chambers of the diluter and that the diluter delivers the appropriate volumes of exposure water to each treatment. During the pretest eyed eggs of a surrogate salmonid species will be exposed to groundwater dilutions containing 11, 24, 54, 120, and 266 $\mu\text{g/L}$ of Cr and a control treatment of Columbia River water. Four replicates of each Cr treatment will be tested.

To initiate the pretest, on day minus 2 the waterbath and exposure chambers will be filled to begin temperature regulation and the groundwater dilutions will be mixed and metered to each treatment. The diluter system will be allowed to equilibrate for 48 hours. On day 0 of the pretest two groups of 50 eyed eggs will be placed into 177-mL glass hatching containers and randomly suspended into each of four replicate exposure chambers per treatment (400 eggs per treatment). The exposure chambers will be covered with black plastic to shield the eggs from light during the exposure and gentle aeration will be used to provide continuous circulation of the exposure water. The sampling regime for the pretest is shown in Table 3.

Table 3. Aqueous chromium sampling regime for Task I onsite ELS pretest.

Days of Exposure	Chromium concentration ($\mu\text{g/L}$) and number of samples						Total
	0.00	11	24	54	120	266	
1	1 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	11 6
3	1 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	11 6
4	1 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	11 6
Total	3	12	6	12	6	12	51

One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder and a 0.4 μm polycarbonate membrane. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle then immediately put on ice and shipped by overnight carrier (or hand delivered) to the analytical laboratory. Upon receipt, the analytical laboratory will immediately conduct ion-exchange separation of the Cr(VI) species. The treated sample containing only the Cr(VI) species will then be acidified to 1% HNO_3 for analysis by ICP-MS. Speciation control checks will include one Cr(VI) spike, one Cr(III) spike and one blank for each

sample collection batch. Spiked concentrations shall not exceed the highest test concentration. The target variation between total Cr and Cr (VI) within the medium and high treatment replicates is $\pm 20\%$ over the duration of the exposure (i.e., mean total Cr and Cr (VI) should not differ by more than 20%). Higher variation may be observed in the low Cr treatments due the limitations of the analytical method. The target variation between nominal and measured concentrations of Cr for all treatments is $\pm 25\%$.

Samples will be analyzed for total Cr with no chemical preparation before instrumental analysis. Methodology used for the analysis of total chromium will follow methods used in the previously completed laboratory ELS exposure. Analysis will be conducted with a PE/SCIEX Elan 6000 ICP-MS or equivalent, which is set up and optimized according to the manufacturer's specifications (see CERC SOP P.241 for operating conditions and quality control procedures). All samples will be prediluted 10X, and any samples over the upper calibration standard of 30 $\mu\text{g/L}$ will be diluted 10X in a serial fashion until concentrations are within the confines of the standard line. The internal standard is Ge (50 $\mu\text{g/L}$), which is metered into the sample line via peristaltic pump. Calibration standards for analysis are 5, 10, 20, and 30 $\mu\text{g/L}$ Cr. One Cr mass is monitored and reported (Cr-52).

Samples collected for chromium speciation will be chemically treated within 24 hours of sampling. Methodology used for the analysis of chromium (VI) will follow methods used in the previously completed laboratory ELS exposure. Ion exchange column chromatography will be used to separate the Cr+3 cation $[\text{Cr}(\text{OH})_2^+]$ from the Cr+6 anion $[\text{HCrO}_4^-]$. Disposable cation exchange columns will be used (AG-50W-X8, hydrogen form) following the procedure outlined in SOP P.454(a). After buffering to pH 5, 20 mL of each sample will be passed through the column (pre-rinsed with 20 mL ultra-pure water) at a flow rate of 1-2 mL/min. The first 10 mL volume will be discarded, and the second 10 mL will be collected in a polypropylene centrifuge tube for the analysis of Cr+6. The collected eluant will be acidified with 0.1 mL of sub-boiled nitric acid and analyses will be conducted as described above for total Cr.

During the definitive on-site test chromium exposure concentrations will be determined with the same methods used for analysis of chromium during the pretest. The minimum sampling regime is shown in Table 4. The frequency or number of samples may be increased based on results of the pretest. For analysis of total chromium, a single, one hundred mL sample will be collected weekly from one replicate within each treatment, and filtered using a Nalgene® 300 filter holder and a 0.4 μm polycarbonate membrane. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3 , and analyzed with ICP-MS (See CERC SOP C5.212). At each time samples are collected for total chromium analysis, one additional sample will be collected from the low, middle, and high chromium treatments, chemically treated, and analyzed for hexavalent chromium (SOP P.454(a)).

Table 4. Minimum aqueous chromium sampling regime for Task I onsite ELS experiment.

		Chromium concentration ($\mu\text{g/L}$) and number of samples						
		0.00	11	24	54	120	266	Total
Weekly	1 total	1 total	1 total	1 total	1 total	1 total	1 total	6
		1 Cr (VI)			1 Cr (VI)		1 Cr (VI)	3
Total	1	2	1	2	1	2		

Aqueous samples for the determination of chromium concentrations will be taken at least daily during the Task II avoidance testing. Samples will be filtered and analyzed for total chromium using the methods described for Task I. Speciation of samples will not be conducted because of the short residence time and duration of the exposure.

4. **Listing of Critical Data:**

Objective 1: to determine the on-site toxicity of chromium-contaminated groundwater from the 100 Area to early life-stages of Columbia River chinook salmon.

Data gathered from this objective will include chinook salmon egg survival and hatching alevin development and mortality, and parr mortality and growth at chromium concentrations representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn.

Objective 2: to determine the avoidance response of chinook salmon parr exposed to aqueous chromium concentrations representative of conditions in the Hanford Reach of the Columbia River.

Data gathered from this objective will include the proportion of time chinook salmon parr spend in chromium-contaminated water representative of conditions in the Columbia River as well as chromium-contaminated up-welling groundwater in the Hanford reach of the Columbia River.

5. **Statistical Treatment:** Percent data for egg, alevin, and parr survival and avoidance time data will be arc sine transformed and analyzed with Analysis of Variance (ANOVA) followed by an appropriate means separation test. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
6. **Acceptance or Rejection Criteria for Results:** Acceptance or rejection criteria for test results are outlined in CERC Standard Operating Procedures and standardized methods

for specific studies. Appropriate Standard Operating Procedures and standardized methods used for these tests are cited in CERC Protocol P00-21-01 (Attachment D).

7. **Special Safety Requirements:** Safety requirements for all research activities at the CERC are given in the CERC Safety Plan.
8. **Endpoint of Study, based on Accomplishments:** This study will provide an assessment of the effects of chromium on the salmon egg survival and hatching, the survival and growth of alevins and parr, and the chromium avoidance response of parr. This study will end with the analysis and interpretation of the data gathered and the submission of a final report to the funding agency, the U.S. Fish and Wildlife Service. Data from this research will also be published in peer-reviewed journals.

9. **Schedule of Study and the Outputs Expected:**

PROJECT SCHEDULE		
ITEM	START	END
Study Design and Organization	Aug 1999	Oct 1999
<u>TASK I: On-site Early Life-stage Toxicity Test</u>		
perform tests	Oct 1999	Mar 2000
data analyses	Apr 2000	Jul 2000
submit draft report	Sept 2000	Oct 2000
submit final report	Oct 2000	
<u>TASK II: Laboratory Avoidance Tests</u>		
perform test	Feb 2000	Apr 2000
data analyses	May 2000	Aug 2000
submit draft report	Oct 2000	Nov 2000
submit final report	Dec 2000	

10. **Place Where Data will be Stored and Archived:** File folders of original data will be maintained at the CERC Columbia, MO according to standard operating procedures. Files from this study will be permanently archived by the CERC QA/QC Officer as outlined in CERC SOP B5.147. Some of the data generated under this project will be stored and archived by Pacific Northwest National Laboratories.
11. **Relationship to Cooperator Needs:** The data gathered from this study will (1) provide an assessment of injury to chinook salmon exposed to chromium in the Hanford area of the Columbia River Basin, (2) will be useful in recovering damages for lost resources and in evaluating remedial options, including immobilization, treatment, and the no-action

alternative; and (3) will be useful to Trustees, including the U.S. Fish and Wildlife Service, Hanford Natural Resource Trustees, the U.S. Department of the Interior, and the States of Oregon and Washington in efforts to manage the Columbia River salmon population.

12. Literature Cited:

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- ASTM (American Society for Testing and Materials). 1993. Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. Philadelphia, Pennsylvania.
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- Dauble, D.D., T.L. Page, and R.W. Hanf, Jr. 1989. Spatial distribution of juvenile salmonids in the Hanford Reach, Columbia River. U.S. National Marine Fisheries Service Fishery Bulletin 87:775-790.
- DeLonay, A.J., E.E. Little, J.J. Lipton, D.F. Woodward, and J. Hansen. 1996. Behavioral avoidance as evidence of injury to fishery resources: Applications to natural resource damage assessments. In: T.W. LaPoint, F.T. Price, and E.E. Little, eds., *Environmental Toxicology and Risk Assessment: Fourth Volume*, ASTM STP 1262, American Society for Testing and Materials, Philadelphia. pp. 268-280.
- Dill, P.A. 1977. Development of behavior in alevins of Atlantic salmon, *Salmo salar*, and rainbow trout, *S. Gairdneri*. *Animal Behavior*, 25:116-121.
- Farag, A.M., C.J. Boese, D.F. Woodward, and H.L. Bergman. 1994. Physiological changes and tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals. *Environmental Toxicology and Chemistry*, 13:2021-2029.
- Farag, A.M., M.A. Stansbury, C. Hogstrand, Elizabeth MacConnell, and H.L. Bergman. 1995. The physiological impairment of free ranging brown trout exposed to metals in the Clark

- Fork River, Montana. Canadian Journal of Fisheries and Aquatic Sciences 52:2038-2050.
- Geist, D.R. 1997. Characterization of spawning habitat within a high and a low density of fall chinook salmon spawning area in the Hanford Reach, Columbia River. Unpublished report by Battelle Pacific Northwest National Laboratory.
- Geist, D.R. 1995. The Hanford Reach: what do we stand to lose? Illahee 2:130-141.
- Geist, D.R., T.M. Poston and D.D. Dauble. 1994. Assessment of potential impacts of major ground water contaminants to fall chinook salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach, Columbia River. Pacific Northwest Laboratory, PNL-9990, Richland, Washington.
- Hansen, J.A., D.F. Woodward, E.E. Little, A.J. DeLonay, and H.L. Bergman. 1999. Behavioral avoidance: A possible mechanism for explaining abundance and distribution of trout species in a metals impacted river. Environmental Toxicology and Chemistry 18:313-317.
- Hartwell, S.I., J.H. Jin, D.S. Cherry and J. Cairns, Jr. 1989. Toxicity versus avoidance response of golden shiner, *Notemigonus crysoleucas*, to five metals. Journal of Fish Biology 35:447-456.
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- Lipton, J.J., E.E. Little, J.C.A. Marr, and A.J. DeLonay. 1996. Use of behavioral avoidance testing in natural resource damage assessment. In: D.A. Bengston and D. Henshel, eds., Environmental Toxicology and Risk Assessment: Fifth Volume, ASTM STP 1306, American Society for Testing and Materials, Philadelphia. pp. 310-322.
- Marr, J., J. Lipton, and D. Cacela. 1995. Fisheries Toxicity Injury Studies Blackbird Mine Site, Idaho. National Oceanic and Atmospheric Administration. Report No. 50-DGNC-1-00007, Washington, D.C.
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salmon at selected study sites in the Hanford Reach of the Columbia River. Report E86-87-3082, U.S. Army Corps of Engineers and U.S. National Marine Fisheries Service. USEPA (United States Environmental Protection Agency). 1986. Quality Criteria for Water 1986. EPA Report 440/5-86-001, Office of Water Regulations and Standards, Washington, D.C.

Wiggins, W.D., G.P. Ruppert, R.R. Smith, L.L. Reed, L.E. Hubbard, and M.L. Courts. 1997. Water Resources Data Washington Water Year 1996. U.S. Geological Survey Water Data Report WA-96-1, Tacoma, Washington.

Woodward, D.F., J.A. Hansen, H.L. Bergman, E.E. Little, and A.J. DeLonay. 1995. Brown trout avoidance of metals in water characteristic of the Clark Fork River, Montana. Canadian Journal of Fisheries and Aquatic Sciences 52:2031-2037.

13. **Signatures:**

Prepared by: _____ Date: _____
Laverne Cleveland
Principal Investigator

Prepared by: _____ Date: _____
Aaron DeLonay
Principal Investigator

Approved by: _____ Date: _____
Edward E. little
Branch Chief

Approved by: _____ Date: _____
I. Eugene Greer
Animal Care and Use Committee Chair

Approved by: _____ Date: _____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date: _____
Wilbur L. Mauck
Center Director

18.0. Appendix B. Curriculum vitae of CERC and PNNL research and administrative/management personnel

RESUME

Employer/Affiliation: Pacific Northwest National Laboratory, Richland, Washington
1973-present

Date Prepared: September 14, 1999

Name: Dennis D. Dauble : _____ Date: _____

Born: Walla Walla, Washington, September 28, 1950

EDUCATION: Oregon State University B.S. Fisheries 1972
Washington State University M.S. Biology 1978
Oregon State University PhD Fisheries 1988

EXPERIENCE:

Technical Group Manager, Ecology Group, 1996-Present

Staff Scientist, Aquatic Ecology Group, 1994-1996

Senior Research Scientist, Environmental Sciences Department, 1988-1994

Research Scientist, Environmental Sciences Department, 1978-1988

Research Technician 1973-1978

PUBLICATIONS:

Dr. Dauble has authored or co-authored over 40 peer-reviewed articles in scientific journals and symposia, >60 technical reports, and a book chapter.

RESUME

Employer/Affiliation Battelle Pacific Northwest National Laboratory
Date Prepared 9/14/99

Name: Gregory W. Patton Signature: _____ Date: _____

Born: Beckley, West Virginia August 25, 1963

EDUCATION:

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
University of South Carolina	Ph.D. Chemistry	1989
Fairmont State College	B.S. Chemistry	1985

TRAINING:

<u>Course</u>	<u>Length</u>	<u>Sponsor</u>
<u>Date</u>		

EXPERIENCE:

Position Title (current), Employer, Location, and Dates.

April 1994 - present: Senior Research Scientist, Environmental Technologies Division, Battelle Pacific Northwest National Laboratory, Richland, WA.

July 1998 - present: Adjunct Lecturer, Washington State University - TriCities, Environmental Science and Regional Planning Program, Richland, WA.

Position Title (next most recent), Employer, Location, and Dates.

December 1989 – April 1994: Research Scientist, Earth & Environmental Sciences Center, Battelle Pacific Northwest Laboratory, Richland, WA.

PUBLICATIONS:

Dr. Patton is the author/coauthor of 8 journal articles, 1 book chapter, and over 20 Pacific Northwest National Laboratory reports. Selected journal articles:

1998. *Strontium-90 in Alfalfa (Medicago sativa) Around the Hanford Site in Southeastern Washington State: An Evaluation of Surveillance Data*, J. Environ. Radioactivity, **39**, 87-105.

1997. *Public Participation in Radiological Surveillance*, Health Physics, **73**, 700-705.

1997. *Ambient Air Sampling for Tritium - Determination of Breakthrough Volumes and Collection Efficiencies for Silica Gel Adsorbent*, Health Physics, **72**, 397-407.

RESUME

Columbia Environmental Research Center

Name: Aaron J. DeLonay _____
Signature Date
Born: Wausau, WI June 5, 1965

EDUCATION:

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
University of Wisconsin-Stevens Point	B.S., Water Resources Aquatic Biology	1987
University of Missouri-Columbia	M.S., Fisheries & Wildlife	1991

TRAINING:

<u>Course</u>	<u>Length</u>	<u>Sponsor /Date</u>
Standard First Aid	4 hours	American Red Cross / 1991
Adult CPR	4 hours	American Red Cross /1992
Driver Safety Training	8 hours	Columbia Safety Council /1992
Natural Resource Damage Assessment	3 days	U.S. FWS/1994
Endangered Species Training	8 hours	U.S. FWS/1996
Boater Safety Training	3 days	U.S. DOI (MSC)/ 1996

EXPERIENCE:

Ecologist, Population Ecology, Midwest Science Center, Columbia, MO, 1993 to present.

Ecologist, Behavioral Toxicology Section, National Fisheries Contaminant Research Center,
U.S. Fish & Wildlife Service, Columbia, MO, 1990 to 1993.

Graduate Research Assistant, University of Missouri, Columbia, MO, 1988 to 1990.

Research Aide, University of Wisconsin-Stevens Point, Stevens Point, WI, September to
December 1987.

Fisheries Intern, Northern Highland Fisheries Research Area, Wisconsin Department of Natural
Resources, Woodruff, WI, May to August 1987.

Teaching Assistant, UW-Stevens Point Treehaven Field Station, Tomahawk, WI, May to August
1986.

PUBLICATIONS: Author or co-author of 12 publications.

RESUME

Columbia Environmental Research Center

Name: William G. Brumbaugh _____

Signature

Date

Born: Painesville, Ohio January 28, 1956

EDUCATION

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
University of Missouri - Columbia	B.S., Chemistry	1978
University of Missouri - Columbia	M.S., Analytical Chemistry	1983
University of Missouri - Columbia	PhD, Analytical Chemistry	1997

TRAINING

<u>Course</u>	<u>Hours</u>	<u>Sponsor</u>	<u>Date</u>
Statistics	6	NFCRC	5/80
Writing Position Descriptions	6	OPM	6/81
Trace Analysis	3 cred.	UMC/NFCRC	9/81
Environmental Chemistry	3 cred.	UMC/NFCRC	9/83
EEO Training	3	OPM	3/84
Advanced Graphite Furnace AA	16	Perkin-Elmer	7/84
Technical Writing	24	Shipley	3/89
Multiculturalism	6	OPM	6/91
Total Quality Management	16	OPM	5/92
Environmental Chemical Analysis	3 cred.	UMC/MSC	9/94
Supramolecular Chemistry	3 cred.	UMC/MSC	1/95
Mass Spectrometry Topics	3 cred.	UMC/MSC	9/95
Organic Spectroscopy Topics	3 cred.	UMC/MSC	1/96

EXPERIENCE:

Leader, Inorganic Chemistry Methods Development, Midwest Science Center, Columbia, Mo, 1990 to present.

Research Chemist, Midwest Science Center (formerly the National Fisheries Contaminant Research Center), Columbia, MO, 1984 to 1990.

Brumbaugh Resume page-2

Chemist, Midwest Science Center (formerly the National Fisheries Contaminant Research Center), Columbia, MO, 1978 to 1984.

Graduate Teaching Assistant, UMC, Quantitative Analysis Lab, 1981.

PUBLICATIONS:

Author or co-author of 24 publications in the field of environmental analytical chemistry including:

1. Brumbaugh, W.G.; Kane, D.A. 1985. Variability of aluminum concentrations in organs and whole bodies of smallmouth bass (*micropterus dolomieu*) *Environmental Science And Technology* 19(9): 828-831.
2. Brumbaugh, W.G.; Koirtzohann, S.R. 1988. Effects of surface on the atomization of lead by graphite furnace atomic absorption. *Analytical Chemistry* 60(10): 1051-1055. (M.S.Thesis, UMC 1983)
3. Brumbaugh, W.G.; Walther, M.J. 1989. Determination of arsenic and selenium in whole fish by continuous-flow hydride generation atomic absorption spectrophotometry. *Journal of the Association of Official Analytical Chemists* 72(3): 484-486.
4. Brumbaugh, W.G.; Walther, M.J. 1991. Improved selenium recovery from tissue with modified sample decomposition. *Journal of the Association of Official Analytical Chemists* 74(3):570.
5. Brumbaugh, W.G.; Ingersoll, C.G.; Kemble, N.E.; May, T.W.; Zajicek, J.L. 1994. Chemical characterization of sediments and pore water from the upper Clark Fork River and Milltown Reservoir, Montana. *Environmental Toxicology And Chemistry* 13(12):1971-83.
6. Brumbaugh, W.G.; Arms, J.A. 1996. Quality control considerations for the determination of acid-volatile sulfide and simultaneously extracted metals in sediments. *Environmental Toxicology And Chemistry* 15:282-285.

RESUME

Columbia Environmental Research Center

Name: Paul R. Heine

Signature

Date

Born: St. Louis, MO May 16, 1949

EDUCATION:

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
University of Missouri, Columbia, MO	B.S., Agriculture	1976

TRAINING:

<u>Course</u>	<u>Length</u>	<u>Sponsor</u>	<u>Date</u>
Training on AAI System	32 hours	Technicon Inc.	1/79
Radiation Safety Training	1 hour	UMC	80
Statistics	1 hour	CNFRL	5/80
Defensive Driving Course	8 hours	Colum. Saf. Council.	6/81
#1 Basic First Aid Course	8 hours	Columbia Chapter	4/82
#2 Basic First Aid Course	8 hours	Columbia Chapter	4/82
Radiation Safety Training	1 hour	UMC	84
Defensive Driving Course	8 hours	Colum. Saf. Council.	12/84
Radiation Safety Training	1 hour	UMC	85
Radiation Safety Training	1 hour	UMC	86
Radiation Safety Training	1 hour	UMC	87
How to Delegate Authority and Ensure It's Done Right	8 hours	Career Track	10/87
Defensive Driving Course	8 hours	Safety Council	12/87
Radiation Safety Training	1 hour	UMC	88
Effective Writing	16 hours	Shiplely Assoc.	3/89
Radiation Safety Training	1 hour	UMC	89
Radiation Safety Training	1 hour	UMC	90
Defensive Driving Course	8 hours	Safety Council	12/90
Hazardous Materials Incident Response Operations	40 hours	EPA, NUS	3/91
Departmental Safety Seminar	40 hours	FWS	4/91

Autoclave Training	1 hour	AMSCO	12/91
Fire Extinguisher Use	1 hour	Nat. Fire Prot. Assoc	12/91
Radiation Safety Training	1 hour	UMC	92
Departmental Safety Seminar	40 hours	FWS	5/92
Hazardous Communication Test		FWS	2/93
Radiation Safety Training	1 hour	FWS	8/93
Departmental Safety Seminar	40 hours	FWS	4/94
HAZMAT Update	8 hours	UMC	5/94
U.S. Coast Guard Boating		USCG	6/94
Radiation Safety Training	1 hour	UMC	8/94
Safe Use of Radioisotopes	20 hours	UMC	2/95
Hazardous Waste Management	24 hours	UMC	3/95
Dept. of Interior Conf.	40 hours	NBS	4/95
OSHA Course #600	40 hours	NBS	4/95
EPA Health & Safety	8 hour	EPA	7/95
Radiation Safety Training	1 hour	UMC	8/95
OSHA Course #600A	40 hours	NBS	9/95
US Dept. Of Interior Boat Operator	40 hours	MSC	6/96
Radiation Safety Training	1 hour	UMC	8/96
Basic First Aid, CPR, Blood Bourne	8 hours	Am. Red Cross	10/96
Laboratory Safety	1 hour	MSC	3/97

EXPERIENCE:

General Biologist, National Fisheries Contaminant Research Center, Columbia, MO, February 1987 to present.

Biological Laboratory Technician (Fisheries), Columbia National Fisheries Research Laboratory, Columbia, MO, 1978 to 1987.

Senior Research Lab. Tech., University of MO. Medical Center, Department of Pathology Chemistry, Columbia, MO., November 1973 to June 1978.

Seasonal Naturalist, MO. State Park Board, Bennett Springs State Park, Nature Interpretive Center, Lebanon, MO, May 1973 to September 1973.

Seasonal Naturalist, MO. State Park Board, Bennett Springs State Park, Nature Interpretive Center, Lebanon, MO, May 1972 to September 1972.

PUBLICATIONS:

Co-author on two publications - SETAC Poster Session 5/18/91.

RESUME

Daniel F. Woodward

NAME: Daniel F. Woodward_____
Signature_____
Date**BORN:** Rolla, Missouri April 19, 1944**EDUCATION:**

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
University of Missouri	B.A., Zoology	1968
University of Missouri	M.S., Fisheries	1970

TRAINING:

<u>Course</u>	<u>Length</u>	<u>Sponsor</u>	<u>Date</u>
Introduction to Supervision	40 hr	OPM	1975
Basic Management Functions	40 hr	OPM	1976
Introduction to Statistics	40 hr	CSU	1977
EEO Training	3 hr	Wm. Walker	1984
Data Analysis, SAS for Micros	16 hrs	CSU	1986

EXPERIENCE:

November 1973 - present. Fisheries Biologist (Research), Project Leader, U.S. Geological Survey, CERC Field Research Station, Jackson, Wyoming. Directs research program to determine the biological effects of aquatic contaminants on cold water fish and aquatic invertebrates of the intermountain area. Responsibility includes all administrative functions, preparation of annual work plans, assigning funds and manpower, preparing an operating budget, and publishing results of the research.

March 1972 - November 1973. Fisheries Biologist (Research) at the U.S. Geological Survey, BRD - Midwest Science Center, Columbia, Missouri. Assistant Leader, Acute Toxicity Section.

January 1970 - January 1972. U.S. Army.

June 1969 - January 1970. Fisheries Biologist, U.S. Geological Survey, BRD-MSC, Tiburon Field Research Station, California.

June 1968 - June 1969. Biological Aid, USGS, BRD, Midwest Science Center, Columbia, MO.

June 1965 - June 1966. U.S. Forest Service, Missoula, Montana

PUBLICATIONS: Principal author on 23 and Co-author on 9 additional scientific publications.

RESUME

Columbia Environmental Research Center

Name: Jesse W. Arms

Born: Independence, MO

Signature

Date

March 13, 1956

EDUCATION:

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
Central Missouri State University - Warrensburg, MO	B.S., Biology	1978

TRAINING:

<u>Course</u>	<u>Length</u>	<u>Sponsor</u>	<u>Date</u>
Technical Writing	5 hours cred.	C.M.S.U.	1981
Organic Chemistry	10 hours cred.	Columbia College	1985
Hazard Materials Shipping	2 days	U.M.C.	1996

EXPERIENCE:

Physical Science Technician, Midwest Science Center (a.k.a. Natl. Fisheries Contaminant Research Center), Columbia, MO, 1986 to present

Analytical Technician, ABC Laboratories, Columbia, MO, 1984 to 1986.

Laboratory Technician, Golden Plant Foods, Concordia-Sweet Springs, MO, 1981 to 1984.

Fieldman, Federal Crop Insurance, USDA, Higginsville, MO, 1980 to 1982.

PUBLICATIONS:

Author or Co-author of 1 publications.

RESUME

Columbia Environmental Research Center

Name: Thomas W. May _____

Signature

_____ Date

Born: Memphis, Tennessee September 5, 1947

EDUCATION

<u>Institution</u>	<u>Degree</u>	<u>Date</u>
University of Alabama	B.S., Chemistry	1969
George Washington Univ.	M.S., Biological Sciences	1975

TRAINING

<u>Course</u>	<u>Hours</u>	<u>Sponsor</u>	<u>Date</u>
Defensive Driving	40	NSC, CSC	1977 - 1990
CPR/First Aid	12	Red Cross	1991 - 1992
Atomic Absorption Training	24	Perkin-Elmer	2/77
Technicon Industries	40	Technicon	6/77
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Patent Procedures and Regulations	4	Don Ralston	1992
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Affirmative Action	3	FWS	3/93
TQM 1	FWS		3/93
TQM Tech and Tools	16	OPM	3/94
HIV/AIDs Training	4	City Health Dept.	2/95

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EXPERIENCE:

Research Chemist, Midwest Science Center, Columbia, MO, 1976 to present.

Laboratory Manager, Dewberry, Nealon, and Davis, Fairfax, VA, 1975 to 1976.

Graduate Teaching Assistant, George Washington University, Washington, D.C., 1972 to 1975.

Physical Science Technician, FBI Crime Laboratory, Washington, D.C., 1970 to 1972.

PUBLICATIONS:

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RESUME

Columbia Environmental Research Center

Name: Laverne Cleveland

Signature

Date

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Education:

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University of Wisconsin, Stevens Point, WI	M.S. Natural Res.	1982

Experience:

Fisheries Biologist, USGS, BRD, Midwest Science Center, Columbia, MO, October 1996 to present.

Fisheries Biologist, National Biological Survey, Midwest Science Center, Columbia, MO, October 1993 to 1996.

Fisheries Biologist, U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, Columbia, MO, 1978 to October 1993.

Selected Publications:

Cleveland, L., Mayer, F.L., Buckler, D.R., and Branson, D.R. 1982. Toxicity of three preparations of pentachlorophenol to fathead minnows - A comparative study. *Environmental Toxicology and Chemistry* 1:205-212.

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Mehrle, P.M., Jr., Cleveland, L., and Buckler, D.R. 1987. Chronic toxicity of an inorganic contaminant mixture to striped bass in fresh and saline waters. *Air, Water and Soil Pollution (Special Issue)* 35 (1-2).

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Nelson, M.K., P.F. Landrum, G.A. Burton, Jr., S.J. Klaine, E.A. Crecelius, T.D. Byl, D.C. Gossiaux, V.N. Tsymbal, L. Cleveland, C.G. Ingersoll, and G. Sasson-Brickson. 1993. Toxicity of contaminated sediments in dilution series with control sediments. *Chemosphere*. 27:1789-1812.

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Cleveland, L., E.E. Little, J.D. Petty, B.T. Johnson, J.A. Lebo, C.E. Orazio, J. Dionne, and A. Crockett. 1995. Toxicological and chemical screening of Antarctica sediments: Use of whole sediment toxicity tests, Microtox, Mutatox, and semipermeable membrane devices (SPMDs). *Marine Pollution Bulletin*. 34:194-202.

Resume
Columbia Environmental Research Center

Edward E. Little, Biologist (Research)

Signature

Date

EDUCATION:

1966-1970	Hiram College, Hiram, OH,	B.A.
Psychology and Biology		
1970-1974	SUNY/Stony Brook, NY	Ph.D.
Biology		

PROFESSIONAL EXPERIENCE:

1978 - 1996, Leader, Behavioral Toxicology Section, National Fisheries Contaminant Research Center, Columbia, MO
1996 - Chief: Ecology Branch, Environmental and Contaminants Research Center

PROFESSIONAL MEMBERSHIPS/RECOGNITION

1998 - Associate Professor (Research), Department of Biological Sciences University of Missouri, Columbia, MO
1998 - Exceptional Service Award American Society of Testing and Materials.
1996- American Society of Limnology and Oceanography
1996- Society of Photobiology
1993 - Adjunct Professor, Institute of Wildlife and Environmental Toxicology. Clemson University. Pendleton, SC.
1991 - Chairman: Subcommittee E47.12: Behavioral Toxicology. American Society of Testing and Materials.
1986 - Society of Environmental Toxicology and Chemistry

PUBLICATIONS:

Environmental Toxicology and Risk Assessment. 1998. Volume 7 Edited by Edward E. Little, Aaron J. DeLonay and Bruce M. Greenberg. ASTM STP 1265. American Society for Testing and Materials, Philadelphia.

Little, E.E. and A.J. DeLonay. 1996 Measures of fish behavior as indicators of sublethal toxicosis during standard toxicity tests. In: *Environmental Toxicology and Risk Assessment: Vol 4*, ASTM STP 1262. Thomas W. LaPoint, Fred T. Price, and Edward E. Little, Eds. American Society for Testing and Materials, Philadelphia. pp. 216-234.

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Zaga, A., E.E. Little, C.F. Rabeni, M.R. Ellersieck. In Press. Photoenhanced toxicity of a

carbamate insecticide to early lifestage amphibians. *Environmental Toxicology and Chemistry*.

Hurtubise, R.D., J.E. Havel, and E.E. Little. **In press**. The Effects of ultraviolet B radiation on Freshwater invertebrates: Experiments with a solar simulator. *Limnology and Oceanography*.

Fabacher, D.L. and E.E. Little. 1996. Skin component may protect fishes from sunburn and fungal infections resulting from exposures to ultraviolet b radiation. *In: Modulators of Fish Immune Responses*. Eds. J.S. Stolen, C.J. Bayne, C.J. Secombes, J.T. Zelikoff, L.E. Twerdoc, D.P. Anderson, SOS Publications, Fair Haven, N.J. 1:241-250.

Hurtubise, R.D., E.E. Little and J.E. Havel. **In press**. Methods for assessing the impacts of UV-B Radiation on Aquatic Invertebrates. *In: Environmental Toxicology and Risk Assessment: Vol 4, ASTM STP 1265*. American Society for Testing and Materials, Philadelphia.

Blazer, V.S., D.L. Fabacher, E.E. Little, M.S. Ewing, and K.M. Kocan. 1997. Effects of Ultraviolet-B radiation on Fish: Histologic comparison of a UVB-tolerant and a UVB-sensitive species. *Aquatic Animal Health*, 9:132-143.

- 19.0. Appendix C. P00-21-01 (Hanford); The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site Toxicity Test and Laboratory Avoidance Tests.

Date Prepared: 8 September 1999

Date Revised: 20 December 1999

Study: P00-21-01; (Hanford); The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site Toxicity Test and Laboratory Avoidance Tests.

This protocol is based on Good Laboratory Practice Standards outlined in the Federal Register (160.120; 40 CFR Part 160, 7/1/85 subpart G - "Protocol for conduct of a study.") This study will comply with all U.S. Geological Survey, Environmental and Contaminants Research Center (USGS/CERC) guidelines for humane treatment of test organisms during culture and experimentation (CERC SOP B5.154, B5.160).

This study protocol is designed to determine the toxicological significance of chromium contamination in the Hanford Reach of the Columbia River. Deviations from this protocol which do not require approval from the Program Chief, Project Manager, Quality Assurance Officer, or the Animal Control and Use Chairman will be recorded in the laboratory notebook. If there is a question on the need for approval, the decision will be made through consultation with principal investigator, supervisor, and the parties above.

A detailed Quality Assurance Project Plan will be prepared for this project and submitted for NRTC review and approval.

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1. Title and purpose of study:

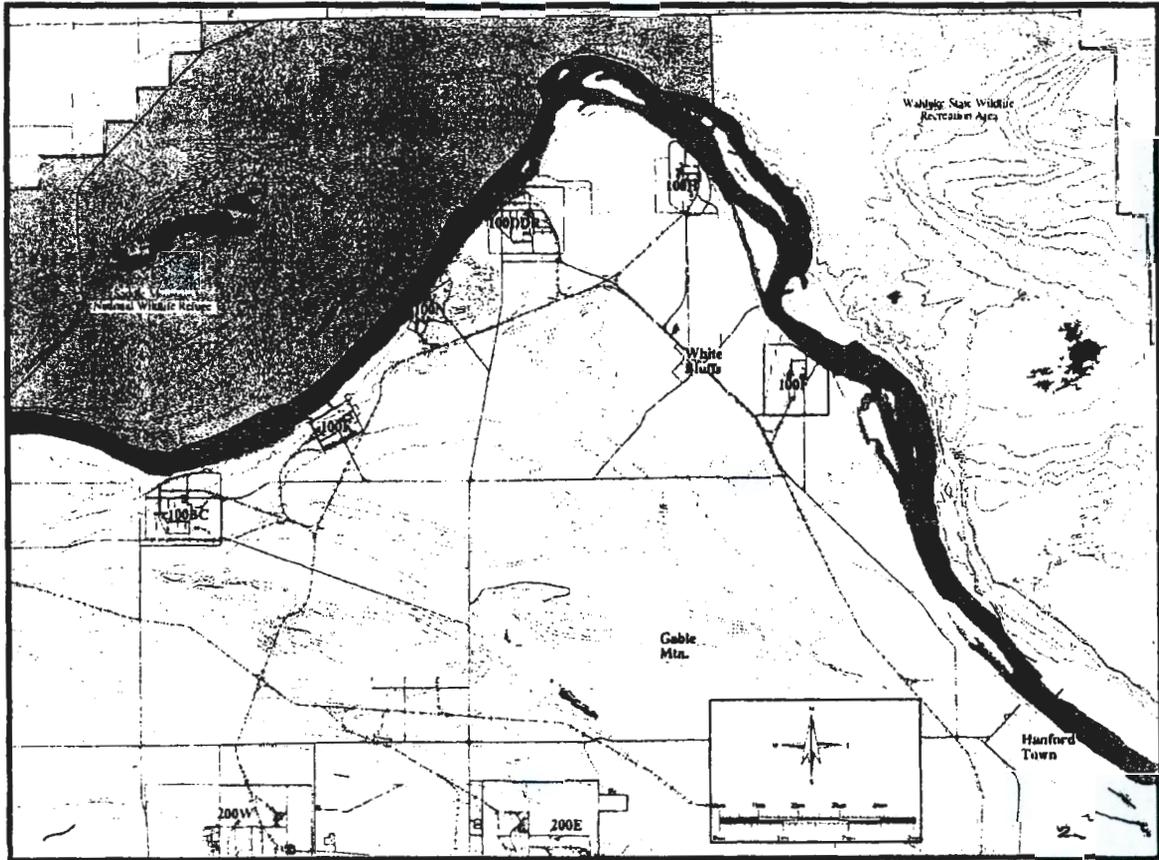
Title of Study: P00-21-01; (Hanford); The Potential for Chromium-contaminated Groundwater from the Hanford 100 Area to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA: On-site Toxicity Test and Laboratory Avoidance Tests.

The Hanford Nuclear Reservation in south central Washington is a 900 square kilometer area claimed by the federal government in 1943 as a site for the production of plutonium (Figure 1)(Geist 1995). The location was ideal because it was remote, sparsely populated, and most importantly, had a readily available supply of cold water from the Columbia River. Because of national security concerns, public access and river development projects were restricted until 1971 (Dauble and Watson 1997). Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Oncorhynchus tshawytscha*). The 90 km section within the Hanford Reservation was not developed and today, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem spawning occurs in the Columbia River (Dauble and Watson 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the mainstem Columbia River.

Large quantities of Columbia River water were used to cool nuclear reactors and cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within the pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the river and also entered ground water through leakage of pipes and seepage from retention areas. Today, groundwater at the Hanford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994). The hydraulic heads of the ground water aquifers in the 100 Area (National Priority List Site) are higher in elevation than that of the Columbia River resulting in discharge from the aquifers into the Columbia River through the river bottom, and shoreline springs and seeps (Figure 1). The ground water is hydraulically connected to the river with peak aquifer discharges occurring during low river flows (fall and winter) and minimum aquifer discharges occurring during high river flows (spring and summer) (Geist et al. 1994).

The use of the Hanford Reach for fall chinook salmon spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook salmon runs have declined during the same time period. Spawning occurs in close proximity to the 100 Area where contaminated ground water is entering the river. Adult chinook salmon spawn in variable water depths, water velocities, and substrate types (Swan et al. 1988). Spawning in the Hanford Reach begins in mid-October, peaks in mid-November, and ends in late November (Dauble and Watson 1997). Egg and fry development within the redds takes place from mid-October to May during low river flows that result in peak aquifer discharges. Based on the mid-November peak abundance of redds and ambient temperatures, eggs would become eyed in early December, hatch in late December, and alevins would emerge from the redds in late

Figure 1. Map of Hanford Reach of the Columbia River. Crosshatched river areas indicate salmon spawning sites.



February. Upon emergence, fry move out of the main river channel into shallow, slow moving, near shore and backwater habitat (Dauble and Watson 1990, Dauble et al. 1989). Juveniles remain in the Hanford Reach from February to mid-July feeding on macroinvertebrates (Becker 1973). Outmigrating begins in May and is usually completed by July at 5-7 months of age, 60-70mm in length, and 3-4 gm in weight (Olson and Foster 1956).

Chromium is a contaminant of major concern and it is associated with groundwater and seeps in the 100 Area. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria (AWQC, 11 $\mu\text{g/L}$) for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA 1986) and the water quality standard for chronic exposure (10 $\mu\text{g/L}$) established by the State of Washington (WAC-173-201A-040). While some data exist on the effects of hexavalent chromium on salmon (Olson and Foster 1956, Buhl and Hamilton 1991), previous studies did not investigate the direct effects on fertilization, effects on alevin exposure only, recovery of exposed alevins, physiological impairment, or behavioral avoidance. Identification of effects and the range of concentrations at which those effects may be manifest is necessary to evaluate the potential for chromium discharge to cause injury to salmon populations in the Columbia River. The Department of Energy currently has activities underway to pump and treat chromium at the Hanford facility, and reduce the amounts of hexavalent chromium released into the Hanford Reach. However, the critical nature of the Hanford Reach as spawning habitat for the chinook salmon makes it essential to determine if current water quality standards protect chinook salmon (Geist 1997). In particular, additional information is needed to determine if the current standards protect early life-stage survival and development.

The early life-stages of chinook salmon are most likely to come in constant contact with elevated chromium and these stages have been shown to be the most sensitive to contaminants (McKim 1977). Chromium may hamper fertilization success by directly acting on the fertilized egg to cause death of the embryo (Billard and Roubaud 1985), or chromium may react with the sperm and egg individually to impede fertilization. If fertilization is successful, chromium may affect the survival of early lifestages (Olson and Foster 1956, Benoit 1976). While it has been documented that elevated concentrations of chromium reduce survival (Buhl 1991), and to a lesser extent, growth (Olson and Foster 1956, Benoit 1976), information has not been gathered on the relevance of recovery periods on these toxicological effects. In the Hanford Reach, chromium that moves from the ground water upwellings becomes diluted extensively. Thus, as young fry begin to emerge from the redds, they may no longer be exposed to elevated concentrations of chromium. The effects of chromium exposure to alevins, as monitored by post-exposure recovery of fry during later development, will mimic the exposure situation present in the Hanford Reach.

Chinook salmon will be present in the Hanford Reach for 5-7 months, and it is important to understand health effects as related to chromium exposure. It is unclear what the exposure concentration might be through contaminated surface water or diet, but long-term health effects from continuous exposure is not well understood in either early life or parr stages (Geist et al. 1994). An understanding of the physiological responses (pathology) associated with chromium exposure can be used to supplement fish population or water and sediment monitoring. Evaluations based on the residue concentrations and physiological condition (e.g. increased lipid peroxidation) of fish integrate the actual exposure to pollutants (dose) and effects

of these exposures on fish survival and growth (Farag et al. 1994, Farag et al. 1995). Further associations of tissue chromium accumulation, oxidative stress, and growth reduction would add more weight to a determination of fish health impairment. This weight of evidence approach uses all of the information gathered to determine the health status of a fish population.

During 1999 we investigated the effects of chromium on salmon exposed during early development and parr stages. The effects of chromium on survival, growth, and parr health were evaluated under controlled conditions in laboratory toxicity tests. Columbia River on-site toxicity tests will be conducted during the year 2000 to more fully characterize the effects of chromium on salmon stocks from the Hanford reach. Also, during 2000 laboratory avoidance tests will be conducted. The goal of these tests will be to determine whether chinook salmon parr exhibit an avoidance response under laboratory conditions to chromium concentrations present in the Hanford Reach of the Columbia River. Fish may avoid concentrations of contaminants well below those levels which may cause mortality or reductions in growth (Little et al. 1985). Avoidance of elevated concentrations of environmental contaminants can alter the distribution of fish in the field and affect habitat use, intra-specific competition, growth and mortality (Woodward et al. 1995, DeLonay et al. 1996, Lipton et al. 1996, Hansen et al. 1999). Chromium avoidance thresholds reported in the literature for other species are within the range of concentrations expected to occur in the Hanford Reach of the Columbia River. Anestis and Neufield (1986) reported an avoidance threshold of 28 $\mu\text{g/L}$ for rainbow trout (*Onchorhynchus gairdneri*) exposed to aqueous chromium. An avoidance threshold level of 73 $\mu\text{g/L}$ chromium has been reported for golden shiners (*Notemigonus crysoleucas*) (Hartwell et al. 1989). Documentation of laboratory avoidance may indicate the potential for chromium contamination to adversely impact habitat quality and availability for early life-stage chinook salmon in the Hanford Reach of the Columbia River.

The objectives of the year 2000 studies will be accomplished in two tasks:

Task I. (On-site Early Life-stage Toxicity Test); Determine the effects of chromium-contaminated groundwater on the hatching success, survival and growth of Columbia River chinook salmon.

Task II. (Laboratory Avoidance Tests); Determine the avoidance response of chinook salmon parr exposed to aqueous chromium concentrations representative of conditions in the Hanford Reach of the Columbia River.

2. Identification of the test and control substance:

In the on-site toxicity test, the test substance will be chromium-contaminated groundwater. Groundwater will be diluted with Columbia River water from the Hanford Reach to obtain the desired chromium exposure concentrations. The control substance in the on-site test will be Columbia River water from the Hanford Reach. Contaminants other than chromium may be present in groundwater and river water at trace concentrations.

In laboratory avoidance tests, the test substance will be chromium as sodium dichromate prepared from a reagent grade stock. The stock will be dissolved in deionized water and metered into the experimental water. Control substance will be the experimental water

without chromium added.

3. Name and address of sponsor and name and address of testing facility:

The sponsor of this study will be the U.S. Fish and Wildlife Service, Upper Columbia River Basin Field Office as part of an interagency agreement (IA) with the U.S. Department of Energy, Richland Operations Office (RL). Work under this IA is being directed by RL and the Hanford Natural Resource Trustee Council, including participation by the U.S. Department of the Interior, U.S. Department of Commerce, U.S. Department of Energy, the Yakima Nation, Confederated Tribes of the Umatilla Indian Reservation, Nez Perce Tribe, and the States of Oregon and Washington. Task I of this study will be performed on-site (Hanford) by the Pacific Northwest National Laboratory, Richland, Washington. Task II of this study will be performed at the testing facility at the U.S. Geological Survey (USGS), Columbia Environmental Research Center (CERC), Columbia, Missouri.

4. Proposed starting and completion dates:

PROJECT SCHEDULE		
ITEM	START	END
Study Design and Organization	Aug 1999	Oct 1999
<u>TASK I: On-site Early Life-stage Toxicity Test</u>		
perform tests	Oct 1999	Mar 2000
data analyses	Apr 2000	Jul 2000
submit draft report	Sept 2000	Oct 2000
submit final report	Oct 2000	
<u>TASK II: Laboratory Avoidance Tests</u>		
perform test	Feb 2000	Apr 2000
data analyses	May 2000	Aug 2000
submit draft report	Oct 2000	Nov 2000
submit final report	Dec 2000	

5. Justification for selection of test system:

Procedures described in ASTM Guide E 1241-92, "Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes" (ASTM, 1993), applicable CERC Standard Operating Procedures (SOPs), and references cited in this Protocol will be used to conduct the test.

6. Species number, body weight, sex, source:

See Section 8, Experimental design.

7. Procedure for identification of test system:

The test system was selected based on review of the available literature on the Hanford

Reach of the Columbia River and as a result of consultations with personnel from the Hanford Natural Resource Trustee Council.

8. Experimental design:

GENERAL. During the on-site toxicity test (Task I) chinook salmon will be exposed to a series of dilutions of chromium-contaminated groundwater from 100 Area (Hanford). Columbia River water from the Hanford Reach will be used to prepare the dilutions. The groundwater source-well will be selected to minimize the potential toxicity due to associated contaminants, other than chromium, that may be present in the groundwater. Concentrations of chromium in the well water should be sufficient so that mixing with Columbia River water does not result in unreasonable deviations from ambient water quality conditions in the river, or unacceptable variation in water quality variables among treatments. Experimental water quality variables should be measured with sufficient regularity (a minimum of once weekly) to document the range of conditions in the Columbia River.

Experimental water used in laboratory avoidance tests (Task II) will be reconstituted to simulate conditions in the Hanford Reach of the Columbia River (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as CaCO₃; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water will be prepared by blending laboratory well water (pH 7.0, hardness 283 mg/L as CaCO₃) with deionized water produced by reverse osmosis. Experimental water will be blended in 5,600 L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. A simulated groundwater will be reconstituted in the same manner, adjusting hardness to 200 mg/L as CaCO₃.

Experimental water temperatures for both tasks will approximate seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Water temperatures gradually increase from late winter through summer. Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Photoperiod will be adjusted to simulate time of year of the exposure.

The chromium concentrations tested in Task I will range from 0 to 266 µg/L. Chromium concentrations tested in Task II will range from 0 to 120 µg/L. This range of concentrations are at or above the chronic AWQC for chromium, 11 µg/L (USEPA 1986). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Geist 1997, Hope and Peterson 1996). Specific concentrations are stated with each task.

Eyed embryos of chinook salmon for the on-site toxicity test will be obtained from Priest Rapids, Washington. Eggs are collected from wild-caught salmon and no disease-free brood stock are maintained in culture. Eggs will be transported to the on-site testing facility and acclimated to exposure conditions before beginning the exposures. The on-site test will begin with eyed embryos and continue through 30 days swim-up.

Eyed embryos for the avoidance test will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to shipment from the hatchery. The disease free status assures that toxicity testing is conducted on healthy

test organisms, increases the reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995). The eyed eggs will be maintained in a Heath^R incubator at a temperature of $10 \pm 2^\circ\text{C}$ and hardness of approximately 280 mg CaCO_3/L . Mortalities will be documented and removed daily. At hatch, the fish will be moved to flow-through culture tanks. The fish will be fed a commercial biodiet daily *ad libitum*. The experimental phase will begin during the parr stage.

TASK I: ON-SITE EARLY LIFE-STAGE TOXICITY TEST:

The purpose of this task is to determine the on-site toxicity of chromium-contaminated groundwater to early life-stage chinook salmon from the Columbia River. Eyed eggs of chinook salmon will be exposed to chromium concentrations 11, 24, 54, 120, and 266 $\mu\text{g}/\text{L}$. The chromium concentrations will be obtained by diluting 100% groundwater with the appropriate volumes of ambient Columbia River water. The control treatment will be 100% ambient Columbia River water. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system (CERC SOP F20.E18, "Construction, Operation, Calibration, And Maintenance of the Proportional Diluter"). Temperature will be maintained at $5 \pm 2^\circ\text{C}$ by chilling the exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the median swim-up date.

During the exposure, egg mortality and hatching will be monitored and recorded daily. Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. Visual observations will be conducted daily to monitor the development of alevins. The sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright plough, free swimming, and exogenous feeding will be documented following Dill (1977).

The following samples will be collected during the experiment for measurements of fish health. This assessment of physiological impairment will be performed if funds become available. Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at median hatch, approximately at the midpoint between hatch and swim-up, swim-up, and termination (30 days median hatch date). Samples will be frozen with liquid nitrogen, and stored at -90°C for later measurements of DNA strand breakage, lipid peroxidation, and tissue chromium measurements. Three fish from each replicate will be collected at swim-up and termination, individually preserved in 10% neutral buffered formalin, and held for histological analyses. The number of samples collected for fish health are listed in Table 1. Poor hatching success or high mortality may require intermediate sampling dates to be eliminated or the number of samples to be reduced. Changes in sampling frequency or number will be made in

consultation with project coordinators. At the end of the exposure (30 days post swim-up) an external necropsy assessment will be made on all surviving alevins, and lengths and weights will be recorded. Fish will not be fed for 24 h prior to sampling.

Table 1. Total number of whole-fish samples collected during Task I on-site ELS test.

TASK I - Onsite Early Life-stage Toxicity Test							
SAMPLE DAY	CONCENTRATION ($\mu\text{g/L}$)						TOTAL
Median Hatch	0	11	24	54	120	266	
Tissue metal, DNA, LP ¹	4	4	4	4	4	4	24
Mid-point Between Hatch and Swim-up							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Swim-up							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Histology	12	12	12	12	12	12	72
Termination							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Histology	12	12	12	12	12	12	72

¹LP = Lipid peroxidation

Total tissue metal, DNA, and LP for Task 1 = 96

Total histology for Task 1 = 108

TASK II: LABORATORY AVOIDANCE TESTS:

The goal of this task is to assess the potential for chromium to influence fish distribution and habitat availability in the Hanford Reach of the Columbia River. The first step in this assessment is to determine whether chinook salmon parr exhibit an avoidance response to chromium under controlled laboratory conditions. Laboratory tests measure the response of salmon presented with a choice between a control condition and chromium-contaminated water; a potentially adverse stimulus. Precisely controlled conditions are necessary to ascribe the observed behavioral response to the presented stimulus. Water quality that simulates the conditions occurring in the Columbia River will be used to control variables such as hardness and pH that are known to affect the speciation, complexation, biological availability and toxicity of metals, such as chromium. Although test conditions will attempt to simulate many of the conditions experienced by fish in the field, the focus of laboratory tests will be to control the nature of the stimulus (aqueous chromium) and the conditions under which it is presented to the organism.

Two avoidance experiments will be conducted. The first will determine the avoidance

response of chinook salmon to chromium concentrations ranging from 0 to 120 $\mu\text{g/L}$ in reconstituted, experimental water (80 mg/L hardness as CaCO_3 , $10 \pm 2^\circ \text{C}$). Selected concentrations were based on the chronic EPA ambient water quality criteria for chromium (11 $\mu\text{g/L}$), concentrations that are expected to elicit an avoidance response based on a survey of the literature, and the range of concentrations expected in the Hanford Reach of the Columbia River.

The second series of avoidance tests is designed to examine whether the water quality characteristics associated with a groundwater source will alter the response of chinook salmon to chromium. Chromium is associated with seeps and areas of upwelling, contaminated groundwater that exist along the river's edge, and in the river bed. Water quality characteristics associated with upwelling groundwater may alter the avoidance response by either changing the perception or toxicity of the stimulus (chromium), or by presenting water quality conditions (hardness, alkalinity, pH, etc.) which may be preferred over the control condition. The second experiment will evaluate the response of chinook salmon to a simulated groundwater (200 mg/L hardness as CaCO_3) with and without the addition of aqueous chromium. The test combinations for each experiment are illustrated in Table 2.

Juvenile chinook salmon (0.25 to 2.0 grams) will be acclimated to, and maintained in experimental water (80 mg/L hardness) at the test temperature ($10 \pm 2^\circ \text{C}$) for a minimum of two weeks prior to the start of avoidance experiments. Fish will not be fed 24 hours prior to testing. Avoidance tests will be conducted using a counter-current apparatus in accordance with CERC SOP B5.232. A Control (experimental water without chromium) and a test solution (experimental water with chromium) flow in from opposite ends of the apparatus, and exit from six drains at the center. This apparatus produces a steep, central gradient between the control and test treatment. Prior to the start of the experiments the apparatus will be calibrated and the steepness of the gradient verified using fluorescein dye.

Individual fish will be placed into each of three avoidance apparatus. After sufficient acclimation (indicated by exploratory behavior) the test will begin. Acclimation times are species and life-stage dependant and usually range from 20 to 40 minutes. The test period will be 40 minutes in duration. The behavioral response to the gradient will be recorded on video tape and analyzed in ten minute intervals as the proportion of time spent in the test solution versus the time spent in the control solution. A series of trials will be conducted using three apparatus, each presenting test organisms with the control and one of the test conditions. This series of treatment combinations will be replicated a minimum of eight times. The test condition delivered to each test apparatus, and the end of the apparatus receiving the test condition will be randomized and alternated between trials. The control combination is a test in which experimental water without contaminants flows into both ends of the apparatus.

Tests will be discarded if there is a disturbance to the avoidance apparatus; inconsistent water chemistry, temperature, or quality; or disease, aggression, or abnormal behavior. Tests will also be discarded if test organisms do not cross the gradient a minimum of three time during the test period. The apparatus will be enclosed in a structure to shield against external movement or sound. Water quality characteristics (pH, alkalinity, hardness, and conductivity) of the reconstituted Columbia River water and Hanford groundwater will be sampled daily.

Table 2. Experimental matrix of treatment combinations used for Task II avoidance tests.

Experiment I	
Control Condition	Test Conditions
Experimental Water ¹ + 0 μg/L Cr	Experimental Water + 0 μg/L Cr
	Experimental Water + 11 μg/L Cr
	Experimental Water + 27 μg/L Cr
	Experimental Water + 54 μg/L Cr
	Experimental Water + 120 μg/L Cr
Experiment II	
Control Conditon	Test Conditons
Experimental Water + 0 μg/L Cr	Simulated Groundwater ² + 0 μg/L Cr
	Simulated Groundwater + 11 μg/L Cr
	Simulated Groundwater + 27 μg/L Cr
	Simulated Groundwater + 54 μg/L Cr
	Simulated Groundwater + 120 μg/L Cr

¹Experimental Water (80 mg/L hardness as CaCO₃)

²Simulated Groundwater (200 mg/L hardness as CaCO₃)

ANALYSIS OF WATER (ALL TASKS):

Prior to conducting the definitive on-site Hanford toxicity test (Task I), a 96-hour flow-through test will be conducted to assess stability of chromium species (e.g., total Cr vs. Cr (VI) exposure concentrations). The procedures for pretest will be identical to those used to conduct the definitive on-site test except that test duration will be shorter (96-h) and the exposure water will be sampled more frequently for analysis of total Cr and Cr (VI). The diluter system will be calibrated before beginning the pretest. The calibration is performed to ensure that appropriate amounts of the groundwater are delivered to the chemical mixing chambers of the diluter and that the diluter delivers the appropriate volumes of exposure water to each treatment. During the pretest eyed eggs of a surrogate salmonid species will be exposed to groundwater dilutions containing 11, 24, 54, 120, and 266 μg/L of Cr and a control treatment of Columbia River water. Four replicates of each Cr treatment will be tested.

To initiate the pretest, on day minus 2 the waterbath and exposure chambers will be filled to begin temperature regulation and the groundwater dilutions will be mixed and metered to each treatment. The diluter system will be allowed to equilibrate for 48 hours. On day 0 of the pretest two groups of 50 eyed eggs will be placed into 177-mL glass hatching containers and randomly suspended into each of four replicate exposure chambers per treatment (400 eggs per treatment). The exposure chambers will be covered with black plastic to shield the eggs from light during the exposure and gentle aeration will be used to provide continuous circulation of the exposure water. The sampling regime for the pretest is shown in Table 3.

Table 3. Aqueous chromium sampling regime for Task I onsite ELS pretest.

Days of Exposure	Chromium concentration ($\mu\text{g/L}$) and number of samples						Total
	0.00	11	24	54	120	266	
1	1 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	11 6
3	1 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	11 6
4	1 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	2 total	2 total 2 Cr (VI)	11 6
Total	3	12	6	12	6	12	51

One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder and a 0.4 μm polycarbonate membrane. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle then immediately put on ice and shipped by overnight carrier (or hand delivered) to the analytical laboratory. Upon receipt, the analytical laboratory will immediately conduct ion-exchange separation of the Cr(VI) species. The treated sample containing only the Cr(VI) species will then be acidified to 1% HNO₃ for analysis by ICP-MS. Speciation control checks will include one Cr(VI) spike, one Cr(III) spike and one blank for each sample collection batch. Spiked concentrations shall not exceed the highest test concentration. The target variation between total Cr and Cr (VI) within the medium and high treatment replicates is $\pm 20\%$ over the duration of the exposure (i.e., mean total Cr and Cr (VI) should not differ by more than 20%). Higher variation may be observed in the low Cr treatments due the limitations of the analytical method. The target variation between nominal and measured concentrations of Cr for all treatments is $\pm 25\%$.

Samples will be analyzed for total Cr with no chemical preparation before instrumental analysis. Methodology used for the analysis of total chromium will follow methods used in the previously completed laboratory ELS exposure. Analysis will be conducted with a PE/SCIEX Elan 6000 ICP-MS or equivalent, which is set up and optimized according to the manufacturer's specifications (see CERC SOP P.241 for operating conditions and quality control procedures). All samples will be prediluted 10X, and any samples over the upper calibration standard of 30 $\mu\text{g/L}$ will be diluted 10X in a serial fashion until concentrations are within the confines of the standard line. The internal standard is Ge (50 $\mu\text{g/L}$), which is metered into the sample line via peristaltic pump. Calibration standards for analysis are 5, 10, 20, and 30 $\mu\text{g/L}$ Cr. One Cr mass is monitored and reported (Cr-52).

Samples collected for chromium speciation will be chemically treated within 24 hours of sampling. Methodology used for the analysis of chromium (VI) will follow methods used in the previously completed laboratory ELS exposure. Ion exchange column chromatography will be used to separate the Cr+3 cation [Cr(OH)₂+] from the Cr+6 anion [HCrO₄-]. Disposable cation exchange columns will be used (AG-50W-X8, hydrogen form) following the procedure outlined in SOP P.454(a). After buffering to pH 5, 20 mL of each sample will be passed through the

column (pre-rinsed with 20 mL ultra-pure water) at a flow rate of 1-2 mL/min. The first 10 mL volume will be discarded, and the second 10 mL will be collected in a polypropylene centrifuge tube for the analysis of Cr+6. The collected eluant will be acidified with 0.1 mL of sub-boiled nitric acid and analyses will be conducted as described above for total Cr.

During the definitive on-site test chromium exposure concentrations will be determined with the same methods used for analysis of chromium during the pretest. The minimum sampling regime is shown in Table 4. The frequency or number of samples may be increased based on results of the pretest. For analysis of total chromium, a single, one hundred mL sample will be collected weekly from one replicate within each treatment, and filtered using a Nalgene® 300 filter holder and a 0.4 μm polycarbonate membrane. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3 , and analyzed with ICP-MS (See CERC SOP C5.212). At each time samples are collected for total chromium analysis, one additional sample will be collected from the low, middle, and high chromium treatments, chemically treated, and analyzed for hexavalent chromium (SOP P.454(a)).

Table 4. Minimum aqueous chromium sampling regime for Task I onsite ELS experiment.

		Chromium concentration ($\mu\text{g/L}$) and number of samples						
		0.00	11	24	54	120	266	Total
Weekly	1 total	1 total	1 total	1 total	1 total	1 total	1 total	6
		1 Cr (VI)			1 Cr (VI)		1 Cr (VI)	3
Total	1	2	1	2	1	2		

Aqueous samples for the determination of chromium concentrations will be taken at least daily during the Task II avoidance testing. Samples will be filtered and analyzed for total chromium using the methods described for Task I. Speciation of samples will not be conducted because of the short residence time and duration of the exposure.

9. Diet, solvents:

Fish will be fed a minimum daily ration of 5% of their body weight of a commercial trout diet. No solvents other than Columbia River water and Hanford groundwater will be used in the on-site toxicity test. In the avoidance studies chromium in the salt form will be dissolved in deionized water.

10. Route of administration:

Fish will be exposed through an aqueous solution.

11. Dosage level of control substances:

The control aqueous solution during the on-site test will be Columbia River water from the Hanford Reach. The control aqueous solution for the avoidance tests will be the reconstituted experimental water with no chromium added.

12. Method for measure degree of absorption of test substances by test system:

Metal adsorption to plastic or glass exposure containers is not anticipated and will not be measured directly.

13. Type and frequency of tests, analysis, measurements:

See Section 8, Experimental design, this protocol.

14. Records:

File folders from this study will be stored at the USGS, Columbia Environmental Research Center, Columbia, Missouri. Handwritten material will be stored according to CERC SOP B5.147 and chain of custody procedures will be followed (CERC SOP QA 5.1). Some of the data (e.g., Task I) will be stored at the Pacific Northwest National Laboratory, Richland,

Washington.

15. Date of approval of Protocol:

See "Approved by" entry in this Protocol under Signatures.

16. Statistics:

Statistical analyses will be performed using SAS system software, version 6.11 (SAS Institute Inc., Cary, North Carolina) or SYSSTAT (SPSS, Chicago, Illinois). Survival and growth data from Task I will be analyzed with Analysis of Variance (ANOVA) followed by the appropriate means separation test. If the data do not meet the assumptions of normality and homogeneity and cannot be transformed to do so, non-parametric statistical analyses will be performed. For Task II data, the proportion of time spent in each treatment will be analyzed using ANOVA followed by a LSD means separation test. Values will be arc-sine transformed before analysis. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.

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Attachment I
STANDARD OPERATING PROCEDURES USED AT THE
COLUMBIA ENVIRONMENTAL RESEARCH CENTER (CERC)
COLUMBIA, MISSOURI

(Partial Listing)

- B4.1 Instrument Design, Maintenance, and Calibration - General
- B4.6 The Mettler PL 200 Top-Loading Balance
- B4.9 pH Determination of Aqueous Samples with the Orion^R Model 901 Microprocessor Ionalyzer and Ross^R Model 82-01 Combination pH Electrode
- B4.14 Maintenance and Storage of Ross Combination pH Electrode
- B4.15 Measuring the Conductivity of Aqueous Samples with the YSI Model 35 Conductance Meter and the YSI 3418 Conductivity Cell
- B4.16 Alkalinity: Burette Method
- B4.18 Measuring Dissolved Oxygen with the YSI^R Model 5B4ARC Dissolved Oxygen Meter
- B4.23 Instruction Manual for the Calibration, Maintenance, and Use of the Top Loading Balance (Mettler AE163)
- B4.29 Mettler AE163 Dual Range Pan Balance
- B4.32 The Mettler P 1200 Top-Loading Balance
- B4.47 Operation and Calibration of Rainin Digital Pipettes
- B4.52 Holding Facilities For Cultured Fish
- B5.1 Feed and Animal Care Materials, Specifications, Storage
- B5.2 Documentation of Sample Receipt
- B5.4 Control of Pesticides and Other Environmental Contaminants in Feed Supplies
- B5.7 Acquisition & Receipt of Fish
- B5.12 Labeling Procedure for Reagents and Solutions
- B5.13 Procedures for Keeping Fish Culture and Acclimation Records
- B5.14 Temperature Monitoring of Freezers and Refrigerators at NFCRC
- B5.16 Glassware Washing Procedure for Analytical Biology Section (Rm. 40)
- B5.63 Storage, Handling, and Retrieval of Hand Written Material
- B5.69 Assignment of Animals to Test Systems
- B5.93 Purchasing, Logging in, and Storage of Toxic Chemicals
- B5.94 Checking Out and Handling of Toxic Chemicals
- B5.102 Procedure for Determining the Length and Weight of Fish from Chronic Flow-Through Toxicity Tests
- B5.106 Safety Plan for the Fish and Invertebrate Toxicology Section
- B5.131 Glassware Cleaning Procedure
- B5.132 Equipment Disinfection
- B5.143 Diet for Fishes
- B5.147 Permanent Archiving of Handwritten Material
- B5.148 Humane Disposal of Fish
- B5.154 Humane Procedures for Anesthetization and Handling of Fish for Sampling Purposes
- B5.160 Reporting Deficiencies in Animal Care and Treatment
- B5.161 Maintenance and Replacement of Well Water Filters Located in the Wet Laboratory

- B5.165 Acclimation of Fish to Research Waters
- B5.167 Reverse Osmosis Units - Quality Control Procedures for Sanitizing and Filter Changes
- B5.168 Replacing Exchange Beds for D.I. Water
- B5.232 Method for Measuring Avoidance/attractance Behavior in Fish
- C5.94 Sample Decomposition of Biological Tissue or Sediment for Elemental Analysis Using Microwave Acid Digestion
- C5.163 Determination of Chromium in Water, Tissue or Sediment Samples By Zeeman Furnace Atomic Absorption Spectroscopy
- C5.212 Environmental Sample Analysis Using the Elan 6000 ICP-MS
- P.241 Environmental Sample Analysis Using the ELAN 6000 ICP-MS
- P.454(a) Analysis for Hexavalent Chromium in Water Samples
- QA4.0 Employee Training Records (In-house)
- QA5.1 Chain of Custody
- QA5.20 Tamper Indicating Security Seals for Sample Containers

20.0. Appendix D. Examples of Selected CERC Standard Operating Procedures

Date Prepared: 09/16/87

Date revised: 09/03/97

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INSTRUMENT DESIGN, MAINTENANCE, AND CALIBRATION - GENERAL

1. All equipment used for the generation, measurement, or assessment of data will be of appropriate design and of adequate capacity to fulfill the operations of the protocols and be located to facilitate operation, inspection and maintenance.
2. Equipment involved in the generation and measurement of data shall be tested, calibrated, standardized, inspected, cleaned and maintained after each use and/or on a routine basis.
3. A written standard operating procedure outlining methods, materials, and schedules used in the routine inspection, cleaning, maintenance, testing and calibration will accompany the equipment. Manuals from the manufacturer can be used with proper identification of their location and any changes in the manuals noted.
4. Equipment SOP's shall specify the action to be taken in the event of a malfunction.
5. Equipment SOP's will designate by title or function personnel who are responsible for its operation.
6. A log shall be maintained near the instrument during the length of the study giving a history of its maintenance, inspection, testing, and calibration. Routine or narrative operation shall be noted and any corrections of malfunctions will be explained and the dates of their occurrence logged in.

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ALKALINITY: BURETTE METHOD

General.

The alkalinity of an aqueous sample is a measurement of its capacity to neutralize acid. The alkalinity of a sample is the measurement of the sum total of titratable bases present. In surface waters, carbonates, bicarbonates, and hydroxyl groups primarily contribute to alkalinity. To a lesser extent, boron, phosphates, silicates, and other ions may contribute to alkalinity.

The measurement of alkalinity is based on the incremental addition of acid to a solution until an arbitrary inflection point in the titration curve is reached. The inflection point of a titration curve is the point at which the shape of curve changes from concave to convex or vice versa. Two inflection points are commonly associated with the measurement of alkalinity; 8.3 (phenolphthalein alkalinity) and 4.5. This document describes the method for determining the alkalinity of a sample to pH 4.5 with a burette.

Required Equipment.

- A. One calibrated pH meter
- B. Calibrated burette (graduated to 0.1 ml)
- C. Magnetic stirrer and stir bar

Required Solutions.

- A. 0.02 N sulfuric acid

Procedure for Determining Alkalinity.

- A. Calibrate pH meter with 4 and 7 pH buffers (see CERC SOP B4.56).
- B. Fill burette to zero mark with 0.02 N sulfuric acid.
- C. Add 100 ml of sample to clean beaker.

- D. Place magnetic stir bar in sample and gently stir sample with magnetic stir plate
- E. Place calibrated pH probe in stirred sample and wait for stable reading. CERC SOP B4.16
- F. Add 0.02 N sulfuric acid to sample from burette slowly until pH 5.8 is reached.
- G. Add 0.1-ml increments of acid to sample until pH 5.0 is reached.
- H. Add 0.02 N sulfuric acid dropwise until pH 4.5 is reached.
- I. Record total volume of 0.02 N sulfuric acid added to sample to reach pH 4.5.
- J. Calculate total alkalinity as ppm CaCO₃ by multiplying the volume of 0.02 N sulfuric acid required to reach a pH of 4.5 times 10.
- K. Record calculated alkalinity value for sample
- L. Remove and rinse electrode with RO water.
- M. See CERC SOP B4.56 for pH meter and electrode maintenance and storage.

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Reference:

SOP B4.56 "Combination pH Electrode, Ross Sure-Flow Model 81-72 Preparation, Maintenance and Storage"

Date Prepared: 09/28/87

For users other than NFCRC staff, this document is for reference only. This is not a citable document.

Date Revised:

FEED AND ANIMAL CARE MATERIALS, SPECIFICATIONS, STORAGE

General: Animals used by NFCR's Aquatic Toxicology Section include several species of fish and invertebrates such as rainbow trout, bluegill sunfish, fathead minnows, channel catfish and *Daphnia magna*. The successful culture of these organisms requires set feeding regimes and periodic prophylactic and therapeutic disease treatments. The following information describes the standard operating procedure employed at NFCR for the storage of feed and animal care materials.

Commercial Fish Foods: All fish species receive a commercially prepared dry high-protein fish food. A series of grain sizes are purchased ranging from fine floating particles to a coarse sinking pellet ration. Fish food is purchased in 50 pound bag lots. With the addition of new food, old food remaining in the storage containers is discarded. The new food shipment is stored in a walk-in cooler at +5°C. When the food volume becomes low in any storage container, new batches of the appropriate food is retrieved from the cooler.

Special Fish Foods: Brine Shrimp - Occasionally, new lots of fish have difficulty adapting to commercial diets. This is particularly the case for fish obtained from open-pond hatcheries where they feed primarily on natural foods. To aid in the transition from natural to artificial food, the commercial diet is supplemented by live brine shrimp which are obtained in the egg form from commercial suppliers and stored dry in their shipping vials until use. When brine shrimp are needed, they are hatched in a salt-water solution and live individuals are transferred to the fish culture system. The procedure used by NFCR in handling brine shrimp is described in Attachment A.

Daphnia Food: *Daphnia magna* receive an artificial diet recommended by the Federal Environmental Protection Agency. The food preparation is stored in a refrigerator at 1°C, with portions removed as needed. The refrigerator is located in a section of the laboratory which is separate from daphnia culture and test systems.

Animal Care Materials: Fish at NFCR receive prophylactic disease treatments upon receipt and specific therapeutic disease treatments as needed. Chemicals used in these treatments include, oxytetracycline hydrochloride, formalin, betadine, $KMnO_4$, and sodium chloride. The refrigerator in which these chemicals are stored is separated from fish culture and test systems and the fish food storage area. Disease treatments are outlined in Attachment C.

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Attachment A

Hatching of Brine Shrimp Eggs

1. Put approx. 3000 mls. of H₂O in a clear glass, one gallon bottle.
2. Put 20 mls. of brine shrimp eggs and 80 mls. of plain salt (no iodine) into a beaker and pour into the H₂O.
3. SHAKE WELL!
4. Place bottle in a water bath at 30°F and using a airstone, moderately bubble air into the H₂O for 48 hrs.
5. After 48 hrs. pour the H₂O + brine shrimp into a separatory funnel that has had the top 3/4 taped or covered to keep out light.
6. Let the separatory funnel set until the brine shrimp have settled to the bottom (10 min.). Then draw off the brine shrimp into a beaker and feed. (The dark colored dead brine shrimp eggs at the bottom should be discarded).

Attachment C

Prophylactic and Therapeutic Treatments
for Freshwater Fish used at NFCR

Disease	Chemical mg/l	Concentration	Application
External bacteria	KMnO ₄	2-4	60 min
Monogenetic trematodes, fungi, and external protozoa	Formalin	150-250	30-60 min
	Potassium permanganate	2-6	30-60 min
	Sodium chloride	15000-30000 2000-4000	5-10 min dip
Eggs upon arrival	Betadine	100	10 min
Parasitic copepods	Trichlorfon (Masoten [®])	0.25 AI	

Date Prepared: 09/18/87

Date Revised: 09/03/97

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TEMPERATURE MONITORING OF FREEZERS AND REFRIGERATORS AT CERC

General:

- A. The monitoring of the large walk-in freezer and refrigerator will be carried out by the temperature sensors located within and connected to the Sonitrol security system.

Procedure:

- A. If it is deemed necessary for daily documentation of temperature levels, a log book will be established and date, temperature, time of day, and person recording the temperature will be recorded in a bound log book.
- B. Recordings of temperatures of the above units will be performed by a member of the research staff..
- C. All temperature records will be kept in the Q.A. files.

Prepared by:

Paul Heine
 General Biologist Date

Approved by:

Jim Fairchild Date
 Ecologist, Community Ecology

Ed Little
 Branch Chief, Ecology Date

Linda Sappington Date
 Quality Assurance Officer

Date Prepared: 09/30/88

Date Revised: 09/19/97

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HUMANE PROCEDURES FOR ANESTHETIZATION AND HANDLING OF FISH FOR SAMPLING PURPOSES

General:

During toxicity tests periodic sampling may be necessary to determine toxicant-induced effects on fish. The humane treatment of experimental fish in laboratory and field investigations insures quality test animals as well as complies with the guidelines and regulations in the Animal Welfare Act and CERC's Animal Welfare Plan.

Procedure:

- A. Disinfect nets and glass or polyethylene sampling containers with Sanaqua (see SOP B5.132). Label these containers for test chambers to be sampled. Remove fish from exposure concentration (sampling from low to high concentration) using a clean net and place into sampling container holding control exposure water. Handling should be accomplished as carefully and quickly as possible to avoid stress.
- B. Water temperature in the sampling containers should be maintained within + 1°C of the test water temperature while measurements are being taken. To maintain temperature, sample containers can be placed in a waterbath or larger container with ice chilled water, or warm water.
- C. The accepted procedure for anesthetization of fishes at CERC is with tricaine methanesulfonate (MS-222). This chemical agent is an acid, and if used in extremely soft water, (hardness less than 10 mg/L as CaCO³) can reduce the pH of the solution to levels (pH of 5 or less) that will stress fish. Check the pH of the solution and add a buffering agent if needed. This anesthetic solution should be changed frequently to ensure that there is sufficient oxygen.
- D. Place only one test fish into sample container that will be used to anesthetize the fish. Carefully dissolve enough MS-222 into this container filled with control water to cause the fish to lose equilibrium and turn on its side but still ventilate.

Note: Many times fish in the higher concentrations are weak and under stress. Add MS-222 slowly and sparingly when anesthetizing these fish. What will anesthetize a control fish may kill a fish from the higher concentration exposure levels. When a safe and effective level of anesthetic has been determined, 3 to 5 fish may be anesthetized and sampled at the same time.

E. Remove the anesthetized fish by carefully grasping the caudal fin with clean forceps. Without causing injuries, quickly and carefully blot fish and place on the measuring board. The standard, fork, or total length of the fish is recorded to the nearest millimeter.

F. Place a large disposable weigh boat containing control water on the balance weighing pan. Tare out the weight of the boat and water. Carefully place the fish into the weigh boat and record the weight to the nearest milligram. Remove the weigh boat and gently pour the fish into the container of fresh control water. These procedures will need to be accomplished with speed and accuracy to prevent the fish from being exposed to the anesthetic any longer than necessary. Wait until all fish have recovered before returning them to the appropriate test chamber.

G. Carefully, gently, and quickly pour fish back into the test chambers. Check back periodically to make sure fish have recovered fully. If there are injuries or mortalities record them on the comment sheets or log book for the test diluter.

Prepared by:

Eugene Greer Date
Leader, Fish Culture Section

Approved by:

Christopher Ingersoll Date
Branch Chief, Toxicology

Linda Sappington Date
Quality Assurance Officer

References:

SOP B5.132 "Equipment Disinfection"

Date Prepared: 01/31/89

Date Revised:

REPORTING DEFICIENCIES IN ANIMAL CARE AND TREATMENT

The humane treatment of experimental animals in laboratory and field investigations is an utmost concern from both a scientific and ethical standpoint. NFCRC staff and members of the general public who observe deficiencies in care or treatment of fish and other vertebrate species or who question procedures in the various research activities have the right to report their concerns to the NFCRC Animal Care and Use Committee (ACUC). The ACUC is obligated to review these concerns and to address them in a timely manner.

If an individual has a question or alleges a deficiency, the concern can be expressed to any ACUC member. Preferably, concerns should be communicated in writing, but a verbal account will carry just as much weight. Include in the report the date, time, place, and the activity which warrants review. The ACUC will review the details and is obligated to file a written report within 15 working days. The ACUC will try to resolve any deficiencies noted through the research supervisory chain and final decisions will be made by the Director of NFCRC. Major deficiencies noted and confirmed will be reported to the Regional Director, Region 8, through the Director of NFCRC. Files of deficiencies reported and action taken to correct them will be maintained by the chairman of the Animal Care and Use Committee. These files may be examined by interested members of the staff or the general public during Center working hours.

Prepared by:

Eugene Greer
Fishery Biologist

Approved by:

Paul M. Mehrle
Chief Biologist

James W. Hogan
Quality Assurance Officer

Date Prepared: 3/8/89
Date Revised: 09/19/97

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ACCLIMATION OF FISH TO RESEARCH WATERS

When fish lots have passed the necessary quarantine period after acquisition, lots may be pulled from the culture tank and transferred to an acclimation reservoir. Any fish being transferred to a water quality different than the culture water in which they are normally maintained must be acclimated to the new water quality and new test temperature over time.

Fish should be acclimated to the research exposure water gradually by changing the water in the acclimation tank from 100% holding water to 100% dilution water over a period of 2 days or more; and they should be acclimated to the new water temperature by increasing/decreasing the temperature approximately 1°C per hour - no more than 8°C per 24 hours. Always aerate the fish acclimation tank to maintain suitable dissolved oxygen levels during the acclimation period. Alternate procedures should be delineated in the appropriate study protocol if deviations from methods stated in this procedure are planned. Standard Operating Procedure B5.13 Procedures for Keeping Fish Culture Records contain details on using the Fish Acclimation Record Form and this form should be used whenever appropriate.

Be sure that all nets and containers used to transfer the fish have to be disinfected with Sanaqua (see SOP B5.132). Handling should be accomplished as carefully and quickly as possible to avoid stress or injury.

Prepared by:

Eugene Greer Date
Fishery Biologist

Approved by:

Christopher Ingersoll Date
Branch Chief, Toxicology

Linda Sappington Date
Quality Assurance OfficerCERC

References:

- SOP B5.13 "Procedures for Keeping Fish Culture Records"
- SOP B5.132 "Equipment Disinfection"

Date Prepared: 29 October 1991

For users other than NFCRC staff, this document is for reference only. This is not a citable document.

METHOD FOR MEASURING AVOIDANCE/ATTRACTANCE BEHAVIOR IN FISH

General

Certain chemical substances can be aversive or attractive to aquatic organisms. Attractance/avoidance responses often mediate exposure to contaminants and provide the only experimental means of assessing the risk of exposure in freely mobile organisms. The ability to detect and avoid a contaminant provides a means of mitigating hazardous exposure, even though avoidance can involve displacement from preferred habitats to areas that are less optimal in terms of shelter, food, or protection from predators. Loss of populations in the field could result from avoidance responses, therefore avoidance provides an important measure of ecosystem injury resulting from the release of hazardous substances. Avoidance/attractance tests measure spatial selection in relation to a gradient of contaminant applied in the water column. The responses to contaminant gradients include preference for uncontaminated or contaminated areas, or a change in spatial preference during contaminant application relative to preexposure preferences. The response measured may include movements into or away from contaminated areas, duration of time spent in contaminated space, frequency of entry into contaminated space, or differences in the speed and patterns of movement in contaminated and uncontaminated space.

Acclimation

Fish shall be acclimated to the temperatures of test control water at a rate of change not to exceed 2° C per day. Acclimation to test control hardness shall not exceed greater than two 50% dilutions in hardness per day over the hardness range of 50 to 300 mg/L hardness as CaCO₃.

Fish should be held under acclimation conditions for at least 72 hours prior to testing.

Fish shall be deprived of food for a minimum of 12 hours prior to test, but shall not be deprived of food for a period greater than 24 hours.

Fish selected for testing shall be in good apparent condition, free of obvious disease, injury, or distress. Fish shall not receive treatments for disease required, in accordance with standard fish culture practices, upon receipt of shipment of eggs or fish.

Apparatus

A cylindrical plexiglass counter-current chamber (approximately 11 cm diameter x 92 cm long) is used to create a contaminant gradient. Control and test solutions enter the chamber from each end, and drain from six drains in the center.

Head tanks receiving control and test solutions from a diluter or from a storage tank of premixed solutions provide constant flow to the chamber through gravity flow or low volume electric pump. Flow into the chambers is regulated by a pair of two-way solenoid valves which, in the rinse mode, will provide uncontaminated water to the chamber, and in the test mode, will supply the experimental solution and control water.

A second pair of switching solenoids directs the flow of test solution to one end of the chamber and control solutions to the other. When activated, the switching solenoid valves will reverse the flow of control and test solutions to each end of the chamber. Activation of the switching valves is achieved through a toggle switch or programmable controller and provides randomization of the contaminant presentation in the chamber to control for position bias.

The bottom of the chamber is covered to provide a uniform, opaque background and overhead fluorescent lighting provides uniform illumination of the chamber to eliminate shadows.

The chambers are surrounded by a partition to prevent visual disturbance of the organism. If necessary, acoustical foam may be used to minimize background sounds.

As necessary, the chamber and/or water supply may be placed in shallow water bath or covered with insulation to maintain constant temperature.

An overhead video camera is used to view the chamber and all studies are video taped. The rinse/test status of the chamber and the position of the test solutions in the chamber are encoded by a 3-LED signal panel. An illuminated central LED indicates a rinsing/acclimation period, whereas LEDs illuminated on either side would indicate the end of the apparatus receiving the test solution. A time readout showing minutes and seconds will be recorded on video tape for later reference during playback of the video recording.

Calibrations

Test chamber calibrations include measuring the rate for flow into each end of the

chamber during rinse and test conditions as well as before and after switching. Water flows should not be less than 750 ml per minute and should not vary by more than 10 % between rinse and test phases, or from one end of the chamber to the other.

Temperatures of acclimation water, and of rinse, control, and test solutions should be within 0.4°C and should not vary more than 0.4°C during an avoidance test.

Other water quality variables such as pH and hardness should be within 10% of the target range.

Water levels within the avoidance chamber should not vary more than 10 % during an avoidance test.

Fluorescein dye calibrations with a spectrofluorometer must be conducted when the chamber is modified, flow rates less than 750 ml/min are used, or average water level in the chamber is greater than 2 cm from the top drain.

Avoidance Test Procedures

Diluter is activated or experimental solutions are mixed in stock tanks, water temperatures are verified, then the control and test solution head boxes are filled.

Avoidance apparatus is filled with rinse water of appropriate temperature and water quality. Temperature is monitored in the head tanks at the beginning and end of the acclimation period and at the beginning and end of the test period. Water temperature is monitored in the avoidance chamber prior to the introduction of the fish and at the end of the test. Water levels within the chamber are monitored throughout the test.

Overhead lights and video camera are turned on. Test date, species, test solution and concentration are listed on a note card which is placed within the field of view of the camera, to identify the avoidance test.

Two juvenile fish are taken from acclimation tank and added to the avoidance chamber. Fish that appear physically or behaviorally abnormal are discarded. Fish that are chased in excess of 2 seconds prior to netting, or handled roughly are not used.

The testing area is enclosed with the partition and the fish are acclimated for 20 minutes, during which the fish arior, territorial behavior, or abnormal responses. Fish showing abnormal responses at this point should be removed and another group selected for test.

ONCE FISH ARE PLACED IN THE CHAMBER AND THE SYSTEM IS ENCLOSED, PARTICULAR CARE MUST BE TAKEN TO AVOID NOISE, VIBRATION, OR VISUAL MOVEMENTS THAT MIGHT DISRUPT THE BEHAVIORAL ACTIVITY OF THE FISH. THE SYSTEM MUST REMAIN CLOSED FOR THE DURATION OF THE TEST.

A 5 minute video sample is recorded during the last five minutes of the acclimation period.

The position where the test solution shall enter is randomly selected, and the switching solenoids are activated as appropriate. The switches are similarly wired and plumbed so as to eliminate the possibility that both solutions could enter the same end of the chamber at once.

The Rinse/Test solenoids are switched 'off' to release the test solutions. The fish are video recorded and monitored in the chamber for 30-40 minutes. This period includes a duration of approximately 5 minutes required for the formation of the gradient and an additional 5 to 10 minute latency period of the fish's reaction

At the conclusion of the 30-40 minute exposure to test solutions in the avoidance chamber, the video recording ends, and the fish are removed from the chamber.

The Rinse/Test solenoids are turned 'on' to rinse the chamber. At flow rates of 750 ml/min, rinsing is completed by 20 minutes.

At the end of the rinse period, another group of fish are selected for the next test.

Data Collection

Data collection can be conducted during the test by viewing the monitor, or by reviewing the video tape at a later date. Even when the data are collected 'live', the session must also be video recorded for future reference.

The video tape is advanced to the beginning of the acclimation footage where the test identification card appears. Information on the card is entered on the transcription sheet and includes; date of test, species tested, nature of test solutions, control water quality, and test temperature. The time readout in the field of view is used to time the experimental sequence and provides an accurate identification of the intervals during which the data are recorded.

The video tape is advanced to the final five minutes of the acclimation period and the

video footage is recorded on the transcription form. At intervals of at least 30 seconds, the number of fish in each side of the avoidance chamber is recorded on the transcription form, along with the time shown in the field of view. The acclimation data may be used for comparisons of pre-exposure and exposure periods, as well as for determining position bias in the chamber.

The video tape is advanced to the onset of the test period, when the center indicator LED is turned off and either of the end LEDs are lighted. The time, video footage, and end of chamber receiving the test solution, as indicated by the lighted LED, is recorded on the transcription form. At 30 second intervals thereafter the number of fish in each side of the chamber is recorded, along with the time of the observation.

Throughout the acclimation and test periods, the behavior of the fish will be monitored for aggressive activity, abnormal swimming patterns, or changes in water flow or water level that might interfere with the fish's spatial selection, and the test will be invalidated and data discarded should such complications arise.

Alternative measured variables

The spatial preference of the fish should be effectively measured at discrete 30 sec intervals, however, when the fish exhibit a high level of activity, with frequent side changes, it may be more effective to count the frequency of entries into one side of the chamber or the amount of time spent on either side of the chamber. Depth of movement into each side of the chamber or relative

swimming speed during entry and exit may also be noted.

Test Replication

Each avoidance test in which the same toxicant quality is presented shall be the experimental unit. The number of test replications shall range from 5 to 20 based on the variance of response.

Data Analysis

The contaminant gradient requires approximately five minutes to reach the target test concentration; therefore, data analysis should not include data obtained during the initial five minutes of the test period. Data will be collected from time 20 minutes to 40 minutes of the test period. It may be desirable to note responses that occur during the initial 20 minutes, as an indication of sensitivity to lower than target

concentrations. The data should be analyzed in five minute segments, with the potential to combine segments in subsequent analyses. The data will be defined as the number of observations in which the fish are present in the side of the chamber receiving the test solution, or the cumulative amount of time spent there. These responses shall be divided by the number of total observations, or total cumulative time of the test to calculate an avoidance ratio. This ratio can also be multiplied by 100 and expressed as percent avoidance. Statistical analyses will be conducted on ratios or cumulative responses from replicate tests.

Rejection of Test Results

Test results will be deemed invalid when abnormal spatial distributions are found to result from social aggression, or abnormal behavior, or by disruption of normal behavior by noise, vibration, or visual stimuli. Malfunction of the apparatus including delivery of test solutions, drainage of the chamber, and failure to control temperatures to within 0.4° C between the ends of the chamber; to within 0.4° C between the test and control solutions; to within 0.4° C between acclimation and test temperatures; or to be in excess of 0.4° C of the target test temperature. Test results may also be deemed invalid if chemical analyses of water quality and test concentrations reveal contaminated control water, or if the pH and hardness vary by more than

10% of specified values.

Sample Collection for Analysis of Test Water

Once during each day of testing, for each concentration applied, water shall be sampled from the end of the chamber receiving the test solution. The samples will be collected at the end of the test period, from the partitioned end of the chamber where the test solution enters the chamber. The samples will be collected, filtered, and preserved as specified in SOP B-5.134. The sample containers will be marked for identification using appropriate study codes.

Disposal of Test Organisms

At the conclusion of the avoidance tests, the fish will be euthanized with 300 mg/L MS-222 for 5 minutes and will be disposed in the sanitary disposal following SOP B-5.148.

Prepared by: .

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Approved by: .

Denny R. Buckler
Chief Biologist

Joseph B. Hunn
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DATE PREPARED 03/23/95

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CULTURE, ANESTHETIZATION, AND EUTHANASIA OF AMPHIBIANS USED IN TOXICITY TESTING

Introduction

This SOP is based on ASTM Guide E 729 - 88a, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" and ASTM Guide E 1439 - 91 "Standard Guide for conducting the Frog Embryo Teretogenesis Assay - Xenopus (FETAX). Amphibians are used as model species in aquatic toxicity studies primarily because of their developmental stages, and their ease of handling, maintenance, and reproduction in the laboratory.

CULTURE

Adult *Xenopus laevis* are obtained from commercial sources and cultured in the laboratory in MSC well water (Hardness, 283 mg/L as CaCO₃; alkalinity 255 mg/L as CaCO₃). The adults are held in flow-through glass aquaria under a 12:12 (light:dark) photoperiod at 20 ± 3 C. The density in the culture tanks should not exceed 4 - 6 frogs/1800 cm² of water surface area. The adult *Xenopus* are fed 3 - 4 times per week with live fish obtained from MSC cultures. Breeding of the adult *Xenopus* to obtain embryos is carried out in the following manner:

1. Adults are bred at 20 ± 3 C under a 12:12 (light:dark) Photoperiod,
2. Males and females are bred as single pairs,
3. Culture water described above is used for breeding,
4. Breeding is induced by injecting males with 250 to 500 IU of Human Chorionic Gonadotropin (HCG) and females with 500 to 1000 IU of HCG. The HCG is injected into the dorsal lymph sac with a 1-ml tuberculum syringe fitted with a ½ inch long 26 gauge needle. The HCG concentration is 1000 IU/ml in 0.9% sterile NaCl,
5. The eggs are dejellied by swirling them for 1-3 minutes in 2% v/v of L-cysteine prepared in culture water adjusted to pH 8.1 with 1N NaOH,
6. The "Atlas of Abnormalities" is used to select normally cleaving embryos for use in the tests, and
7. Midblastula (stage 8) to early gastrula (stage 11) is used to start the FETAX test.

Embryos of *Bufo* sp., *Rana* sp., and *Hyla* sp., are obtained from commercial sources or wild populations. The embryos are cultured in the laboratory under flow-through or static renewal conditions in the well water described above at 18 to 22 C and a

12:12 light:dark) photoperiod. The tadpole stages of these species used in testing are maintained in glass aquaria or other glass chambers. During culture and testing the tadpoles are fed daily with a mixture of ground rabbit chow and fish flakes.

Anesthetization

During the injection of Xenpous with HCG no anesthetization is required. The injections are performed or supervised by trained biologists. The adult Xenpous are restrained in large nylon nets or nylon mess gabs and the injections are done quickly with only minor discomfort to the animals.

Anesthetization to tadpoles (Bufo sp., Rana sp., and Hyla sp.) for growth determinations is accomplished by submerging the tadpoles in a 100 mg/l solution of MS-22 in cultured water. The tadpoles are quickly weighed and measured, placed in cluture water for resuscitation, and then returned to the exposure chambers.

Euthanasia

Larval Xenpous and tadpoles of Bufo sp., Rana sp., and Hyla sp., are euthanized for growth, contaminant tissue residue analyses, and analysis of biochemical endpoints. Euthanization is accomplished by submerging the animals in a 200 mg/l solution of MS-222.

Prepared by:

Laverne Cleveland Date
Fishery Biologist (Research)

Approved by:

Denny R. Buckler Date
Chief Biologist

Linda Sappington Date
Quality Assurance

Eugene Greer
Chairman, Animal Care and Use Committee

Date Prepared: 09/21/87

Date Revised: 09/09/97

For users other than CERC staff, this document is for reference only. This is not a citable document.

PROCEDURE FOR COLLECTING WATER SAMPLES FROM CHRONIC FLOW-THROUGH TOXICITY TESTS FOR RESIDUE ANALYSIS

I. General:

- A. Chronic toxicity tests with fish are typically conducted in proportional flow-through diluters. Exposure water samples are usually taken for residue analysis after fish have been added to the exposure tanks and following a period of operation (at least 24 hours) to ensure that nominal test concentrations have been obtained (day 0 of test). Sampling frequency is dependent on the test duration and nature of the toxicant and is described in the Test Protocol or attachments to the Test Protocol. Parameters such as 1) sample volumes, 2) type of sample containers, 2) type of filters, 3) type and number of reagent blanks, 4) number of spiked and duplicate samples, and 5) sample preservation procedures depend on the chemical characteristics of the toxicant and are determined prior to initiating the test. These parameters are described in the Test Protocol or attachments to the Test Protocol.

II. Sampling Procedure for Non-Filtered Water Samples:

- A. Water samples greater than 100 milliliters are siphoned from the center of the exposure chamber at mid-dept with a clean glass siphon tube. Three tube volumes are discarded to ensure adequate flushing of the tube and the appropriate sample volume is collected in a clean graduated cylinder. Water samples less than 100 milliliters are collected with a clean 100 milliliter glass pipet attached to a Drummond[®] portable pipet-aid. As in the collection of the larger samples, the tip of the pipet is placed in the center of the exposure chamber at mid-dept. All samples are collected in ascending order of exposure concentrations (i.e. controls first up through the highest concentration) to avoid having to wash the sampling tube or pipet between concentrations. The samples are then transferred to the sample containers and labeled with the date, toxicant name, a unique sample identification number, and the collector's initials. The sample is then preserved according to procedures that are described in the Test Protocol or attachments to the Test Protocol.

- B. Filtered samples are collected in the same manner as described above for non-filtered samples. Following collection of the appropriate volumes, samples are transferred to the sample reservoir of a filtration apparatus and filtered under vacuum across an appropriate filter. The filtered sample is then decanted into an appropriate container and preserved.

- C. Residue Analysis Transmittal Sheets (Attachment A) are filled out completely for each set of samples.

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Linda Sappington Date
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Date Prepared: 09/21/87

Date Revised: 09/09/97

For users other than CERC staff, this document is for reference only. This is not a citable document.

PROCEDURE FOR COLLECTING FISH FROM CHRONIC FLOW-THROUGH TOXICITY TESTS FOR TISSUE RESIDUE ANALYSIS

Samples of fish are collected during chronic flow-through toxicity tests to determine the amount of toxicant absorbed by the test organism and the rate at which the organism eliminates the toxicant. Sampling frequency, amount of tissue collected, and preservation techniques depend on the nature of the test and toxicant and are determined prior to initiating the test. These parameters are described in detail in the Test Protocol or attachments to the Test Protocol. Fish tissue samples are collected from chronic flow-through toxicity tests in the following manner.

1. During a chronic flow-through toxicity test, fish are exposed to a series of toxicant concentrations and a control which are replicated. Samples of fish are taken from each replicated exposure concentration for residue analysis.
2. A top-loading scale is used for quick and easy access. The scale is turned on and allowed to warm-up for at least 30 minutes. The appropriate calibration procedures are performed to ensure that the scale is functioning properly. The scale should also be checked periodically with standard weights to ensure its accuracy.
3. Live fish are randomly removed from the exposure chambers with a clean net and placed into clean rectangular glass or polyethylene sampling containers in control exposure water. The number of fish taken per replicate sample depend on the size of the fish and the amount of tissue required to perform the analysis.
4. The fish are dosed with a sufficient amount of tricaine methanesulfonate (MS-222) to cause immediate death.
5. Individual fish from the replicate samples are removed from the anesthetic solution, blotted, pooled, and a wet weight determined to the nearest hundredth gram. The fish are then transferred to an appropriate sample container. Labels

containing the date, a unique sample identification number, test day, species, exposure concentration, weight of pooled sample, and the collector's initials are affixed to the sample containers. Residue Analysis Transmittal Sheets or Chain of Custody forms (Attachment A) are filled out completely for all samples. The samples are then preserved and stored until

analysis according to procedures described in the Test Protocol or attachments to the Test Protocol.

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Linda Sappington Date
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Date Prepared: 09/22/87

Date Revised: 09/19/97

For users other than CERC staff, this document is for reference only. This is not a citable document.

ANESTHETIZATION OF FISHES FOR SAMPLING PURPOSES

I. General:

Tricaine methanesulfonate (TMS, MS-222) has been used for over 50 years to anesthetize fish. TMS has been intensively studied over the past 25 years as to efficacy, toxicity and residues in a variety of fishes. The water concentrations of TMS necessary to induce anesthesia varies with the species of fish used and the water chemistry of the anesthetizing solution.

II. Anesthetic Solution:

A. Because there are differences in the response to TMS, the following concentrations are given as guides with the actual levels to be determined by the species used and the response and time to that response being the final determinants.

<u>Fish</u>	<u>Guidelines are for Rapid Anesthesia*</u>
Salmonids	100-150 mg/l
Escids	100-200 mg/l
Cyprinids	150-200 mg/l
Ictalurids	150-250 mg/l
Centrarchids	200-300 mg/l
Percids	100-150 mg/l

*Loss of reflex activity in 2-5 minutes (see Schoettger and Julin, 1967).

B. TMS is an acid and if used in extremely soft water (hardness less than 10 mg/l as CaCO₃) can reduce the pH of the anesthetic solution to levels that will stress fish (pH of 5 or less). Check the pH of the anesthetic solution and if needed bring to pH 7 with bicarbonate.

C. The anesthetic solution should have sufficient oxygen so as not

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REVISED 05/21/96

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COLLECTION AND PRESERVATION OF WATER AND TISSUE
TO BE ANALYZED FOR TRACE METALS

General: Samples from the environment can be highly variable in character. This variability, in addition to the numerous possibilities of analytical requirements for the sample, makes it difficult to provide a set of guidelines to cover all sample collection and preservation situations. Thus, the following collection and preservation information is offered as a general guideline and is subject to changes to allow adaptation to specific sample characteristics and/or analytical requirements.

WATER SAMPLES

1. Field. Collect two 500 mL samples at each site, one filtered and the other unfiltered, unless study protocol directs otherwise. Recommended materials and acid cleaning procedures are listed in a later section.

(a) non-filtered sample - collect by submersing an acid-cleaned conventional polyethylene bottle into the water using tongs or waterproof gloves; it is a good idea to rinse the bottle out with a small volume of the water to be collected prior to the actual collection; preserve with high purity HNO_3 or HCl (refer to study protocol) by adding enough acid to achieve an effective concentration of 1% v/v. Leave about a 1 inch head space below the cap and seal tightly. Sealed ampules of HNO_3 or HCl are available for convenient field use (see section entitled "Recommended materials, supplies, and other information").

(b) filtered sample - use Geotech filtration unit and follow attached procedures for use and operation. Perform filtration with a Nucleopore 0.40 μm polycarbonate membrane. Use the first few mLs of filtrate to rinse out the sample bottle. After filtered sample is in a pre-cleaned polyethylene bottle, acidify to a 1% effective acid concentration with high purity HNO_3 or HCl (refer to study protocol). Collect enough water to fill the bottle, leaving about a 1 inch head space below the cap and seal tightly.

2. Laboratory. Collect two 100 mL samples from each tank, location, etc., one

filtered and one non-filtered or follow study protocol.

(a) Collect a filtered sample as described in 1(a) above.

(b) Collect Filtered Sample as Above. Smaller Hand-held Filtration Units May Be Used in Place of the Geotech Unit I.e., the Nalgene Polysulfone Filtration Unit (Cole-parmer # N-06730-55). If the Nalgene unit is used, remove the membrane filter between samples and rinse the unit with at least 20 mL of dilute nitric acid (0.1 - 1.0% v/v), followed by an equal volume of high purity water. The unit may be rinsed by use of a squirt bottle to the upper chamber, allowing the rinse to drain through the filter support into the receiver, then swirling and discarding.

(c) Sample water is received from field unfiltered and unacidified. Shake 15 seconds and pour off sample if needed for head space and let acclimate to room temperature. Add 10mL of sub-boiled H₂NO₃ per 1L (1% effective) water. Let set for 24 hours. Shake sample. Filter as in section 2b. This will be the dilute acid extractable.

TISSUE SAMPLES

Collected samples may be placed in either a Whirl-Pak bag or small Zip Lock bag (for small samples), or in large clear polyethylene bags (or large Zip Locks) for the larger tissue samples. Pre-cleaned 4 oz wide-mouth bottles with Teflon®-lined closures (e.g., I-Chem) are recommended for invertebrates, eggs, etc. Larger, whole specimens for compositing (such as whole body fish) should be individually wrapped in plastic (Glad wrap or Saran wrap) before placing in larger bags. When transferring samples to storage containers, care should be exercised to minimize handling of specimens. Where applicable, polypropylene tongs, forceps, or spatulas, should be utilized for manipulation of samples. Specimens which appear to have surface contamination from sediment must be flushed with water from which they were collected. Samples should be put on dry ice or in a freezer immediately.

LABELING

On a dry surface of the water bottle, place a pressure-sensitive label and fill in with an indelible marking pen. Place clear scotch tape over the entire label to waterproof it. For tissue samples, write on the Whirl-Pak bag with an indelible marker; tie a cloth tag around the outer polyethylene bag for the larger samples. Write the sample ID on the cloth tag with an indelible marking pen. Cloth tags 2 5/8" x 5 1/4" are available from the GSA Supply Catalogue.

The following should be included as additional samples with each collection unless the study protocol directs otherwise:

(1) Field reagent blank - distilled or deionized water poured into the sample container and then the appropriate preservative is added. This is a check on the purity of reagents and cleanliness of

container(s). If filtration is performed, deionized water is filtered and preserved.

(2) Duplicate samples - identical samples are taken assigned separate sample numbers, and sent back to the lab as blind samples. This is a check on the precision of analytical results.

(3) Spiked samples (optional) - a known quantity of analyte is added to one of a pair of duplicate samples. This is a check on accuracy of analytical results and possible loss of analyte during storage.

Numbers of the above samples to be taken - a minimum of three reagent blanks for each collection of samples and two duplicate sample pairs per collection of samples. If spikes are included: two spiked sample pairs, one member of a pair spiked with low concentration and one member spiked with a high concentration of the analyte. (Study protocol may include additional guidelines).

TISSUE QUALITY CONTROL

About the only form of quality control that can be exercised with collections of tissue samples is a replicate sample collection. For example, if a group of fish were captured in a net, select several fish of approximately the same species, length, weight, and age. Divide this group of similar fish into two groups. When these two groups are supplied to the lab as two composite fish samples, the contaminant profile should be similar for each sample. If desired, blind tissue control materials, such as NBS Bovine Liver, etc. can also be supplied, but unfortunately most of these materials can be identified on the basis of their appearance, color and odor.

RECOMMENDED MATERIALS, SUPPLIES AND OTHER INFORMATION

Acid Cleansing Procedure - follow MSC SOP C5.5.

0.40 um polycarbonate membrane Filters - Corning Costar, One Alewife Center, Cambridge, Massachusetts 2140 1-800-882-7711, 47 mm diameter, Costar stock

#111107; 142mm diameter, Costar stock #112107 or 112207.

Nalgene polysulfone filter holder; Cole-Parmer N-06730-50.

Geotech filtration unit, available from Geotech Environmental Equipment, Inc.; 1441 West 46th Ave., Unit #17, Denver, Colorado 80211 1-800-833-7958.

Whirl-Pak sampling bags; available from Cole-Parmer Instrument Company, 7425 North Oak Park Avenue, Niles, IL 60714. 1-800-323-4340.

Polyethylene bags of all shapes and sizes, from Associated Bag Company, 400 West Boden Street, Milwaukee, Wisconsin 53207-7120 1-800-926-6100.

Pre - cleaned sample containers,(200 series, polyethylene; 300 series, glass) available from I-Chem, 2 Boulden Circle, Suite B, New Castle, DE 19720-2064 (302) 322-3808

Cloth tag labels of varying sizes, available from GSA Supply

JT Baker Ultrex-II Nitric or Hydrochloric Acid. Available from VWR Scientific.

Sealed ampules of reagent grade HNO₃ and HCl are available from Eagle Picher, 200 B.J. Tunnell Blvd., Miami, OK 74354-3300 1-800-331-7425.

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PROCEDURE FOR ACID CLEANING PROPORTIONAL DILUTERS

GENERAL:

Organisms used in toxicity tests must be disease-free to limit the number of stress factors imposed on them during a toxicity test to only the toxicant(s) being tested. To prevent disease and residual chemical transmittal from one test to another conducted in the same diluter, a rigorous cleaning procedure must be accomplished on the various components of a diluter between tests. This SOP describes the procedure for acid cleaning diluters between toxicity tests at the Yankton Field Research Station.

APPARATUS:

1. Scouring pad.
2. Long-handled brush.
3. One liter beaker.

REAGENT:

1. Fish Pesticide Research Laboratory acid cleaning solution: a combination of HCl (38.2%) and a detergent, commercially prepared.

PROCEDURE:

1. At the completion of a diluter toxicity test, remove all remaining organisms present in the diluter aquaria and discard into the zero-discharge lagoon. Turn OFF the water supply to the diluter. Also, disconnect the power supply, interval timer, cycle alarm timer, Micromedic(s), water circulators, and water chillers, if

present.

2. Wearing the proper safety attire, e.g., rubber gloves, rubber boots, lab coat, and goggles, remove the aquaria plexiglass cover lids and clean with soap and soft water, rinse with deionized water, and allow to air dry. Remove the glass/stainless steel dividers and clean with cleaning acid, rinse well with deionized water and allow to air dry.
3. Remove the waterbath standpipe and drain the waterbath until empty. Remove the aquaria standpipes and allow the aquaria to drain until empty. Place all standpipes into a cleaning acid bath.
4. Carefully place an appropriately-sized rubber stopper into the open end of the delivery line to each aquaria which delivers test water from the flow-splitting boxes (SOP 5.2) to the test system. Using the one liter beaker, fill the flow-splitting boxes on the diluter back with cleaning acid. This acid will drain into the delivery lines. Let acid set in the delivery lines while cleaning the aquaria (step 5).
5. Scrub the sides of each aquaria with a scouring pad and cleaning acid to remove any residual material. After cleaning all aquaria, scrub the flow-splitting boxes with a brush. Then remove the rubber stoppers from the delivery lines and allow the acid to drain into the aquaria.
6. Rinse the flow-splitting boxes and delivery lines thoroughly with soft or deionized water until all traces of soap are removed.
7. Place an appropriately-sized rubber stopper in aquaria drain and the delivery lines. (NOTE: Rubber stoppers are again inserted in the delivery lines to prevent any backsplash of soapy water into the cleaned lines.) Fill each aquaria with soft water and allow to overflow into the waterbath until all traces of soap are removed.
8. After all aquaria have been rinsed thoroughly and drained, vacuum dry with the shop vacuum.

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PROCEDURE FOR PH DETERMINATION - ORION EA 940

A. Equipment and Standards

1. The Ph meter used is an Orion ionalyser model EA940 equipped with a temperature compensating probe Orion 917001 (See Jackson SOP: F20.E10).
2. The probe used is a Ross 81-02 combination glass body electrode 0-14 pH range. The probe is stored in a 200 ml pH 7 standard buffer solution with 1 g of KCl added. The internal filling solution for the probe is 3M KCl.

B. Two Buffer Calibration

1. Two buffers are selected that will best bracket the expected pH sample. Generally they are selected from: pH 4.01, 7.00 and 10.00. For example, if the expected pH is 5.0, then the two buffers selected would be pH 7.00 and pH 4.01.
2. The pH meter can either be calibrated automatically or manually.
3. The Orion EA 940 operates by asking a series of questions on a digital display board. The operator answers these questions by pressing yes or no keys and the meter moves on to the next step.
4. We have found that manual calibration fits our needs best. The following is the series of steps the operator must go through to calibrate the EA 940 and measure pH of sample.
 - The operator presses "speed" key then numeric key "2." The meter then displays "operator menu?"
 - The operator presses "no" key. Then the meter displays "calibrate 1 pH?"
 - The operator presses "yes" key. Meter will display "enter number of buffers."
 - The operator presses "2." Meter will display "automatic calibration?"
 - The operator presses "no" key. The meter will display "1 pH electrode in buffer 1?"
 - The operator then place the electrode and the temperature compensating probe in the pH 7.00 buffer and presses "yes" key. The meter will then read "buffer 1 = 7.06"

cal. as

7.00?"

--Operator then enters the actual pH value of the buffer by pressing the proper numeric keys. (For example if the actual value of the buffer is pH 6.97, the operator enters 6.97).

The meter then displays "buffer 1 = 7.06 cal. as 6.97?"

--The operator presses "yes" key. The meter will then display "1 pH electrode in buffer 2."

--The operator places the electrode in buffer number 2 and presses the "yes" key. The meter then displays "buffer 2= 4.04 cal. as 4.01?"

--The operator enters the actual pH value of the second buffer and presses "yes" key. The meter will then display "Slope 99.5% yes to continue" (Slope must be between 92 and 104%).

--The operator then presses "yes." The meter will display the pH value of the sample.

After the meter is calibrated, electrodes can continuously be placed from sample to sample and the pH value can be read directly from the display board for each sample.

Probes should be rinsed with distilled water and excess moisture blotted off between each buffer and each sample that is measured.

6. Name of operator, date, time, and the buffers used are entered in a log book that is kept by the pH meter after each calibration.

7. PH meter is calibrated a minimum of once each day.

C. pH Readings

1. The pH of exposure tanks are checked daily in each individual tank during the treatment period by taking approximately 200 ml samples in a beaker from the tank. The pH and temperature probes are placed directly into the sample and the reading is taken from the meter display board.

2. Results of the pH reading are then recorded by the operator on a special form attached to the diluter. The tolerance of the pH reading is held within ± 0.2 of the required pH.

D. Automatic Temperature Compensating Probe (ATC)

1. The ATC enables the operator of the EA 940 to samples and buffers at any

