Chemist, Midwest Science Center (fomerly 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 dolomieui) Environmental Science And Technology 19(9): 828-831.

2. Brumbaugh, W.G.; Koirtyohann, 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
Midwest Science Center
March, 1997

NAME: Aïda M. Farag _____ _____
Signature Date

BORN: Gary, Indiana March 26, 1959

EDUCATION:

Institution	Degree	Date
Indiana University	B.S., Envnmtl. Health Mgt.	1981
University of Wyoming	M.S., Zoology & Physiology	1988
University of Wyoming	Ph.D., Zoology & Physiology	1993

TRAINING:

Course	Length	Sponsor	Date
Radioisotope Techniques	40 hr	Univ. of WY	1987

EXPERIENCE:

July 1993 - Present. Fisheries Biologist (Research), Assistant Project Leader, NBS, Midwest Science Center, Jackson Field Station, Jackson, Wyoming. Directs and conducts research both in the laboratory and the field on cold water fish and aquatic invertebrates. Assists the Project Leader with all operations at the field station which include supervising technical staff, preparing study designs, budgets, protocols, standard operating procedures, progress reports, quality assurance plans, laboratory safety documents and scientific manuscripts.

January 1993. Consultant to the University of Wyoming, Dept. of Zoology and Physiology. Prepared draft documents for the U.S. NOAA guidelines for determining injury to fish and wildlife.

September 1991 - December 1993. U.S. Department of Energy Doctoral Trainee. Department of Zoology and Physiology, University of Wyoming, Laramie, WY. Conducted research on the physiological impairment of fish exposed to metals.

September 1985 - September 1991. Graduate Research Associate, Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, WY. Conducted research on the aquatic toxicology of metals, acid deposition, oil field discharges and fuel oil.

1982 - 1985. Research and Volunteer Assistant for various education and government agencies.

Principal or co-author of 14 scientific publications.

RESUME
Midwest Science Center, National Biological Survey
March 25, 1997

Name: Paul R. Heine _____ _____
Signature Date

Born: St. Louis, MO May 16, 1949

EDUCATION:

Institution Degree		Date
University of Missouri, Columbia, MO	B.S., Agriculture	1976

TRAINING:

Course	Length	Sponsor	Date
Training on AAII 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. Counc.	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. Counc.	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	Shipley 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
January 26, 1997

NAME: Daniel F. Woodward _____ _____
Signature Date

BORN: Rolla, Missouri April 19, 1944

EDUCATION:

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

TRAINING:

Course	Length	Sponsor	Date
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. Geiological Survey, BRD-MSC, 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 scientific publications.
Co-author on 9 additional scientific publications.

RESUME
Environmental and Contaminants Research Center
September 9, 1998

Name: Jesse W. Arms

Signature Date

Born: Independence, MO March 13, 1956

EDUCATION:

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

TRAINING:

Course	Length	Sponsor	Date
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
Environmental and Contaminants Research Center
April 3, 1997

Name: Thomas W. May _____ _____
Signature Date

Born: Memphis, Tennessee September 5, 1947

EDUCATION

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

TRAINING

Course	Hours	Sponsor	Date
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
Statistical Analysis	36	UMC	9/79
Analysis of Variance	36	UMC	5/80
Trace Analysis	60	UMC	8/81
Technical Writing	24	Shipley	2/79
Supervision & Group Performance	40	OPM	4/79
Role of Supervisors in EEO	24	OPM	2/80
Writing Job Descriptions	16	OPM	2/82
EEO Training	3	Walker	3/84
Multiculturism	6	Ross and Company	6/91
Radiation Safety Training	1	UMC Reactor	1991
Ethics Regulations	3	T.J. Sullivan	1992
Patent Procedures and Regulations	4	Don Ralston	1992
EEO Training, ManagingDifferences	0.5	GSA	1992
Affirmative Action	3	FWS	3/93
TQM I	FWS		3/93
TQM Tech and Tools	16	OPM	3/94
HIV/AIDs Training	4	City Health Dept.	2/95

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:

Author or co-author of 24 publications.

RESUME
Midwest Science Center
March 27, 1997

Name: Laverne Cleveland

_____ _____
Signature Date

Born: Boyce, LA July 25, 1951

Education:

Institution	Degree	Date
Grambling State University, Grambling, LA	B.A. Biol.	1974
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.

Cleveland, L. and Hamilton, S.J. 1983. Toxicity of the organophosphorus defoliant DEF to rainbow trout, Salmo gairdneri and channel catfish, Ictalurus puntatus. Aquatic Toxicology. 4:3411-355.

Cleveland, L., Mayer, F.L., Buckler, D.R., and Palawski, D.U. 1986. Toxicity of five alkyl-aryl phosphate ester chemicals to four species of freshwater fish. Environmental Toxicology and Chemistry 5:273-282.

Hamilton, S.J., Cleveland, L., Smith, L., Lebo, J., and Mayer, F.L. 1986. Toxicity of pure pentachlorophenol and chlorinated phenoxyphenol impurities to fathead minnows. Environmental Toxicology and Chemistry 5:543-552.

Cleveland, L., Little, E.E., Hamilton, S.J., Buckler, D.R., and Hunn, J.B. 1986. Interactive toxicity of aluminum and acidity to early life stages of brook trout. Transactions of the American Fisheries Society 115(4):610-620.

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).

Cleveland, L., E.E. Little, and R.H. Wiedmeyer. 1988. Chronic no-observed-effect concentrations of aluminum for brook trout exposed in low calcium dilute acidic water. In: Environmental Chemistry and Toxicology of Aluminum, ed. T.E. Lewis. Lewis Publishers, Inc., Chelsea, MI.

Cleveland, L., D.R. Buckler, and W.G. Brumbaugh. Residue dynamics and effects of aluminum on growth and mortality of brook trout. 1991. Environmental Toxicology Chemistry. 10:243-248.

Cleveland, L., E.E. Little, C.G. Ingersoll, R.H. Wiedmeyer, and J.B. Hunn. 1991. Sensitivity of brook trout to low pH, low calcium, and elevated aluminum concentrations during laboratory pulse exposures. Aquatic Toxicology. 19:303-318.

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. Ingersol, and G. Sasson-Brickson. 1993. Toxicity of contaminated sediments in dilution series with control sediments. Chemosphere. 27:1789-1812.

Cleveland, L., E.E. Little, D.R. Buckler, and R.H. Wiedmeyer. 1994. Toxicity and bioaccumulation of waterborne and dietary selenium in bluegill (Lepomis microchirus). Aquatic Toxicology. 27:265-280.

Buckler, D.R., L. Cleveland, E.E. Little, and W. Brumbaugh. 1995. Survival, sublethal responses, and tissue residues of Atlantic salmon exposed to acidic pH and aluminum. Aquatic Toxicology. 31:203-206.

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
Environmental and Contaminants 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.

Little, E.E. and D.E. Fabacher. 1996. Exposure of Freshwater fish to simulated solar UVB radiation:Chapter 8. In G.K. Ostrander, editor, *Techniques in Aquatic Toxicology*, Lewis Publishers, Boco Raton. pp 141-158.
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. *Limonology 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. Stolén, 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. P99-10-01; (Hanford); The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawystcha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA.

JACKSON PROTOCOL P99-10-01

Date Prepared: 8 May 1998

Date Revised: 22 July 1998

Study: P99 -307-10-01; (Hanford); The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawystcha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA.

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, Biological Resources Division, Environmental and Contaminants Research Center (USGS/BRD/ECRC) guidelines for humane treatment of test organisms during culture and experimentation (ECRC SOP B5.160; Jackson SOP F.P02, F.P03).

This study Protocol is designed to determine the toxicological significance of chromium contamination in the Hanford Reach of the Columbia River. In April 1998, justification for these studies was presented and supported by the Hanford Natural Resources Trustee Council (NRTC) Work Group for the Hanford 100 Area Aquatic Assessment. 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 of Principal Investigator, supervisor, and the parties above.

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

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1. Title and Purpose of Study: Title of Study: P99-10-01; (Hanford); The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawystcha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA.

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 (*Ocorhynchus tshawystcha*). 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 Handford 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 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 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

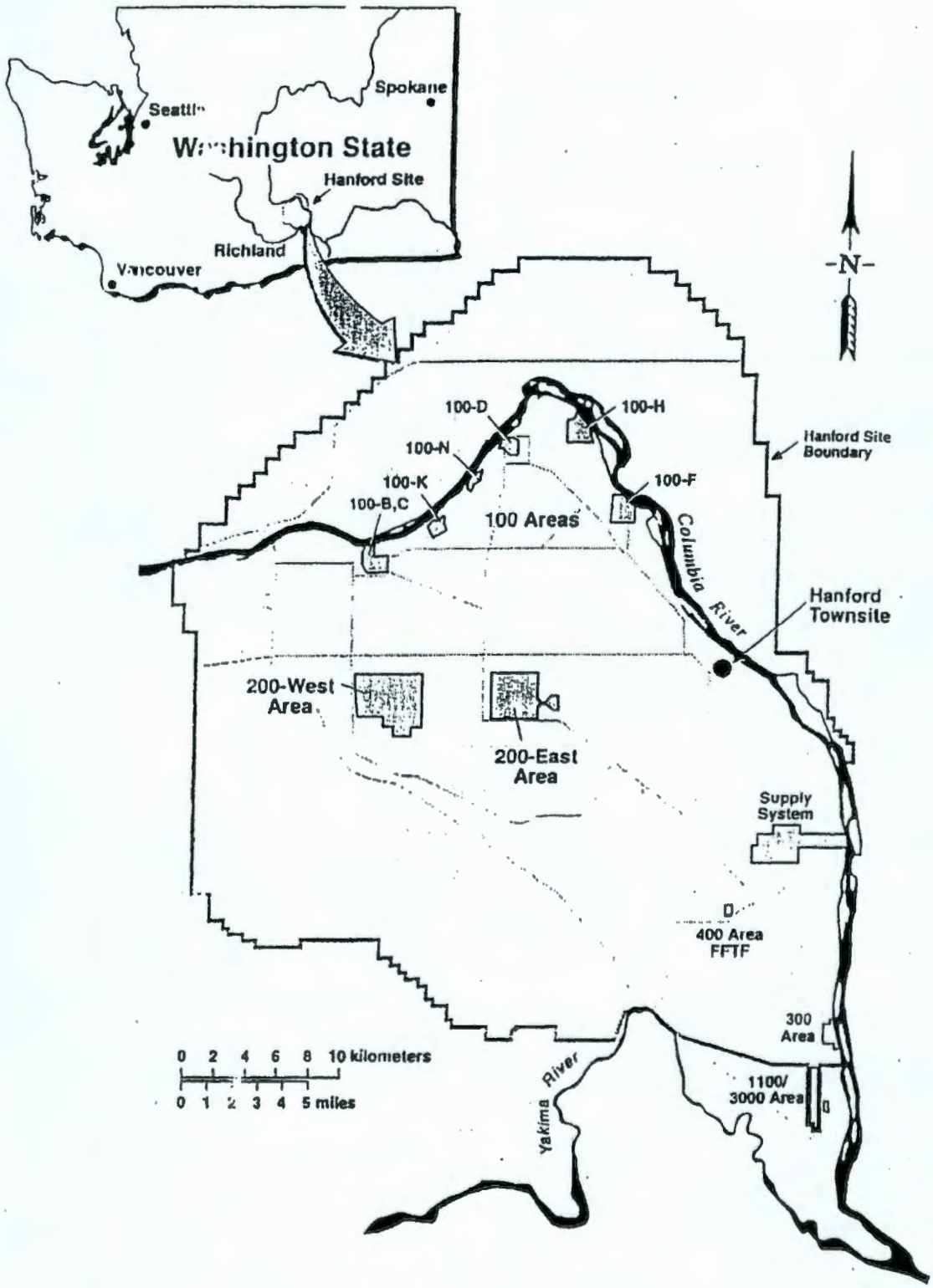

Figure 1. Map of Hanford Reach of the Columbia River

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). One contaminant of major concern, and associated with the 100 Area groundwater and seeps, is chromium. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria (AWQC, 11 μg/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). 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. While some data do exist on the effects of hexavelent 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, or physiological impairment.

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 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.

We will meet two objectives by investigating the health status of salmon exposed to

chromium during the early and parr stages. First, the effects of chromium on survival and growth of chinook salmon will be explained in terms of the health status of individual fish. Additionally, tools can be developed in the laboratory under controlled conditions, that can later be used to assess the health of fish in the Hanford Reach.

The objectives of this proposed 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. This objective will be accomplished in three tasks:

Task 1. (Fertilization); The potential for chromium to adversely affect gametes and their fertilization in chinook salmon.

Task 2. (Early Life Stage); Determine the effects of chromium on the early development of chinook salmon.

Task 3. (Fish Health); Determine degree of fish health impairment of chinook salmon exposed to chromium.

Task 4. (Reports); Draft and final reports.

2. Identification of the Test and Control Substance:

The test substance will be chromium as sodium dichromate prepared from a regent 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 with the Hanford Natural Resource Trustees, including participation by the U.S. Department of the Interior, U.S. Department of Commerce, U.S. Department of Energy, the Yakama Nation, Confederated Tribes of the Umatilla Indian Reservation, Nez Perce Tribe, and the States of Oregon and Washington. This study will be performed at the testing facility at the U.S. Geological Survey, Biological Resources Division, (USGS-BRD), Ecological and Contaminants Research Center (ECRC), Columbia, Missouri and the Jackson Field Station, Jackson, Wyoming.

4. **Proposed starting and completion dates:**

PROJECT SCHEDULE		
ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
TASK 1: Fertilization		
perform tests	Nov 98	Dec 98
data analyses	Jan 99	Mar 99
TASK 2: Early Life Stage		
perform test	Nov 98	Mar 99
tissue analyses	Mar 99	Sep 99
data analyses	Oct 99	Dec 99
TASK 3: Parr Health		
perform tests	Mar 99	Jun 99
tissue analyses	Jun 99	Dec 99
data analyses	Jan 00	Mar 00
TASK 4: Reports		
Submit draft report	Mar 00	Jun 00
Submit final report		90 days after receipt of comments

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 ECRC 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:

Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach and 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 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 (Giest 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

Gametes and 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).

TASK 1: FERTILIZATION:

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 μg/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 $CaCO_3$; 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.

TASK 2: EARLY LIFE STAGE:

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±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 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.

TASK 3: PARR HEALTH:

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 lifestage experiment. Because fry at the end of the early lifestage 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 Gutterirdge 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 ± 2 °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 µg/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 µg/L Chromium, and four with 24 µg/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.

ANALYSIS OF WATER AND TISSUE (ALL TASKS):

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).

TOTAL NUMBER OF SAMPLES:

Table 1.

TASK 2 - EARLY LIFE STAGE							
SAMPLE DAY	CONCENTRATION (µg/L)						TOTAL
Day 0	0	5	11	24	54	120	
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Histology	8	8	8	8	8	8	48
Day 7							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Day 14							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Day 28							
Tissue metal, DNA, LP	4	4	4	4	4	4	24
Histology	8	8	8	8	8	8	48

LP = Lipid peroxidation
Total tissue metal, DNA, and LP for Task 2 = 96
Total histology for Task 2 = 96

Table 2.

TASK 3 - PARR HEALTH					
SAMPLE DAY	TISSUE	CONCENTRATION (µg/L)			TOTAL
Day 0		0	11	24	
Tissue chromium, DNA, LP	Gill	4			4
	Kidney	4			4
	Liver	4			4
	Intestine	4			4
	Whole Body	4			4
Histology	Gill	8			8
	Kidney	8			8
	Liver	8			8
	Intestine	8			8
	Spleen	8			8
	Skin	8			8
Day 60 and 100					
Tissue chromium, DNA, LP	Gill	4 x 2	4 x 2	4 x 2	24
	Kidney	4 x 2	4 x 2	4 x 2	24
	Liver	4 x 2	4 x 2	4 x 2	24
	Intestine	4 x 2	4 x 2	4 x 2	24
	Whole Body	4 x 2	4 x 2	4 x 2	24
Histology	Gill	8 x 2	8 x 2	8 x 2	48
	Kidney	8 x 2	8 x 2	8 x 2	48
	Liver	8 x 2	8 x 2	8 x 2	48
	Intestine	8 x 2	8 x 2	8 x 2	48
	Spleen	8 x 2	8 x 2	8 x 2	48

TASK 3 - PARR HEALTH					
SAMPLE DAY	TISSUE	CONCENTRATION (µg/L)			TOTAL
	Skin	8 x 2	8 x 2	8 x 2	48

LP= lipid peroxidation; Total tissue metal, DNA, and LP for Task 3 = 140; Total histology for Task 3 = 288.

9. Diet, solvents:

Fish will be fed 5% of their body weight on a daily basis with a commercial trout diet. Chromium in the salt form will be dissolved in a water stock solution.

10. Route of administration:

Fish will be exposed through an aqueous solution.

11. Dosage level of control substances:

The control aqueous solution will be the experimental water with 0 chromium added.

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

Metal adsorption to plastic or glass aquaria 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/BRD, Environmental and Contaminants Research Center, Columbia, Missouri and the Jackson Field Station, Jackson, Wyoming. Handwritten material will be stored according to ECRC SOP B5.230 and chain of custody procedures will be followed (ECRC SOP AQ 5.1)

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). Multivariate Analysis of Variance (MANOVA) followed by Tukey means comparisons will be performed on all data that meet the assumptions of homogeneity and normality. The dependent variables include percent fertilization, survival, growth, DNA strand breakage, and lipid peroxidation. The number of replicates for each experiment is four. If the data do not meet these assumptions and cannot be transformed to do so, non-parametric statistical analyses will be performed. 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 To Protocol P99-10-01:
LIST OF APPLICABLE STANDARD OPERATING PROCEDURES

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
C5-212 Environmental Sample Analysis Using the Elan 6000 ICP-MS
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

F20.E18	Construction, Operation, Calibration, And Maintenance of the Proportional Diluter
F20.P15	Procedure for Reconstituting Test Water from RO/DI Water, Raw Water and Salts
QA5.1	Chain of Custody
QA5.20	Tamper Indicating Security Seals for Sample Containers
QA4.0	Employee Training Records (In-house)
F.E01:	Operation and maintenance of the Walter Louis Chemical Water Softener with Aqua Matic Model 514 Regeneration Controller.
F.E02:	Operation and maintenance of the Walter Louis Chemical Reverse Osmosis water filtration unit.
F.E03:	Operation, maintenance, and backwash of the Walter Louis Chemical twin-bed deionization water treatment units.
F.E04:	Operation and maintenance of the sand filtration system for discharge water treatment.
F.E05:	Operation and maintenance of the carbon filtration system for discharge water treatment.
F.E06:	Operation and maintenance of the Culligan mixed bed deionized unit with Sybron/Barnstead purity meter.
F.E07:	Operation and maintenance of the Remcor model CFF liquid circulator, heater/chiller
F.E08:	Operation, calibration, and maintenance of the Model 2500 Micromedic automatic pipette and associated pumps
F.E10:	Operation, calibration, and maintenance of the Orion Model EA 940 Ionanalyzer.
F.E13:	Operation, calibration, and maintenance of the Satorius L42OP electronic top loading scale
F.E14:	Operation, calibration, and maintenance of the YSI Model 54ARC dissolved oxygen meter and probes
F.E15:	Operation and maintenance of the Panasonic Color Video Camera (WV-3250/8AF), Panasonic Video Recorder (AG-2400), Panasonic AC Adapter (AG-B12), and the Panasonic Video Monitor (ST-1000M)
F.E17:	Underground Emergency Toxic Storage Tank
F.E18:	Construction, operation, calibration, and maintenance of the proportional diluter.
F.E19:	Operation am maintenance of the VWR Model 640 manufactured by Amber Science, Inc., Eugene, Oregon
F.E20:	Operation, Calibration, and Maintenance of the Lotek SRX_400 Radio Telemetry Receiver
F.E21:	Operation and maintenance of the REVCO ELITE 500 Model Ulta-cold Freezer.
F.E22:	Operation and Maintenance of the Sensidyne Pump Model 800 and Gastec Precision Gas Detection System
F.E23:	Operation and Maintenance of the Hitachi Model F-2000 Fluorescence Spectrophotometer

F.E24:	Operation and Maintenance of the Bio-Rad Model 3550-UV Microplate Reader
F.E26:	Maintenance and operation of Flowmeter
F.P01:	Culture and handling of test fish
F.P02:	Humane procedures for anesthetization and handling of fish for sampling purposes
F.P03:	Humane disposal of fish
F.P04:	Procedure for determining the toxicity of chemical substances to early life-stages of fishes
F.P07:	Sediment handling for toxicity studies: preventing potential for fish disease contamination
F.P08:	Procedure for measuring water temperature
F.P09:	Procedure for measuring dissolved oxygen in water
F.P10:	Procedure for determining total water hardness
F.P11:	Procedure for determining alkalinity in water
F.P12:	Procedure for pH determination - ORION EA 940
F.P14:	Procedure for conductivity determination
F.P15:	Procedure for Reconstituting Test Water from RO/DI Water, Raw Water and Salts
F.P16:	Operation of Automatic Controllers for Reconstituted Test Water System
F.P19:	Procedure for Dry Diet Preparation at Bozeman from Field collected Fish Food Organisms: Preventing Disease and Assuring Presence of Necessary Vitamins and Minerals
F.P21:	Sampling Procedure for Biofilm and Benthic Macroinvertebrates
F.P22:	Procedure for Force-feeding Fish
F.P23:	Procedure for Collecting Intestinal Fluids from Fish
F.P24:	Procedure for Collecting Water Samples when Sterile Conditions are Required.

ATTACHMENT II To Protocol P99-10-01: PROCEDURES FOR PRETESTING

A. Pretest For Early Life Stage Experiment:

1. Test procedures: 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 Hanford Protocol. The procedures for pretest will be identical to those used to conduct the definitive Hanford Protocol 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). Before beginning the pretest the automatic pipettes used to deliver the sodium dichromate stock solution and the diluter system will be calibrated. The calibrations are performed to ensure that appropriate amounts of the stock solution 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 surrogate salmonid species will be exposed to 5, 11, 24, 54, and 120 μg/L of Cr and a control treatment with no Cr added. Four replicates of each Cr treatment will be tested.

2. Test initiation: To initiate the pretest, on day minus 2 the waterbath and exposure chambers will be filled to begin temperature regulation and the Cr stock solution 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.

C. Sampling regime:

Days of Exposure	Chromium concentration (μg/L) and number of samples						
	0.00	5	11	24	54	120	Total
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

2. Pretest For Parr Health Experiment:

A. Experimental units in study design: 12 experimental units; four each of 0, 11 and 24 μg/L Cr in water treatments.

B. Experimental units will be maintained separately and randomly assigned to 12 holding tanks.

C. Fish will be fed at 2.5% body weight at 8:00am and 11:00am; tanks will be cleaned at 1:00pm; and water will be sampled at 3:00pm.

D. General water quality sampling will be performed as identified in Protocol.

E. Pretest will last 5 days.

1. Day 0: initiate cycling of water through diluter.
2. Day 1: initiate metering Cr.
3. Day 2: sample for total Cr (all tanks) and Cr VI (randomly selected tank of the 11 and 24 ug/L treatment) prior to adding fish
4. Day 2: add fish to tanks at 1 gm/liter
5. Day 3: sample as in Day 2
6. Day 4: sample as in Day 2
7. Day 5: sample as in Day 2
8. Day 5: sample as in Day 2, but just prior to cleaning at 1:00pm.

F. Sampling regime:

Day of Pretest	Chromium concentration (μg/L) and number of samples			
	0.00	11	24	Total
2	4 total	4 total 1Cr (VI)	4 total 1 Cr (VI)	12 2
2	4 total	4 total 1 Cr (VI)	4 total 1 Cr (VI)	12 2
3	4 total	4 total 1 Cr (VI)	4 total 1 Cr (VI)	12 2
4	4 total	4 total 1 Cr (VI)	4 total 1 Cr (VI)	12 2
5	4 total	4 total 1 Cr (VI)	4 total 1 Cr (VI)	12 2
5	4 total	4 total 1 Cr (VI)	4 total 1 Cr (VI)	12 2
Total	24	30	30	84

III. Sample Collection, Analysis, and Targets; Early Life Stage and Parr Health Experiments:

The samples collected for Cr(VI) analysis will be filtered as described in the Hanford Protocol for total Cr, then immediately put on ice (without acidification) and shipped by overnight carrier (or hand delivered) to the analytical laboratory. Upon receipt, the analytical laboratory will immediately conduct extraction or ion-exchange separation of the Cr(VI) species. The extracted sample containing only the Cr(VI) species will then be acidified to 1% HNO3 for analysis by ICP-MS or graphite furnace atomic absorption spectrophotometry. The target variation between total Cr and Cr (VI) within the medium and high treatment replicates is ± 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 ± 25 %.

IV. Validation of Cr and Cr(VI) Sampling and Measurement

A. Analysis Methods: Inductively coupled plasma-mass spectrometry (ICP-MS) or graphite furnace atomic absorption spectrophotometry (GFAAS) will be used for analysis. Each method essentially detects both ionic species of Cr equally, therefore, the analysis for individual species is preceded by a separation procedure. It is anticipated that the optimum analytical method will be dependant on the choice of separation method, i.e., GFAAS determination may be required if an extraction method is utilized because traces of solvent can hinder analysis by ICP-MS. Quality control for all analyses will include duplicates, spikes, reference solutions, and blanks according to EPA-CLP guidelines.

B. Speciation Method: Low pressure column ion-exchange and/or extraction with APDC/MIBK will be evaluated for separation of Cr (III) and Cr (VI). Each method will be assessed based on precision, accuracy, blank values, and species discrimination. A report outlining the performance of the method to be used will be issued before initiation of the pre-testing phase.

C. Cr (VI) Speciation Method Validation: Three simulated test waters (80 ppm hardness) will be prepared including: 1) 100 µg/L Cr(III) as $Cr(NO_3)_3$, 2) 10 µg/L Cr(VI) as $K_2Cr_2O_7$, and 3) 100 µg/L Cr(VI) as $K_2Cr_2O_7$. Each of the three spiked test waters and one blank test water will be determined in triplicate. Each replicate sample (100 mL) will be filtered through a 0.4 µm polycarbonate membrane to simulate actual test water sampling. Recovery checks will be assessed at 0, 24, and 48 hr for Cr(VI) spiked water samples stored at 4°C in order to simulate behavior of shipped samples.

D. Target Values:

1. Cr(VI) mean recovery: 90 - 110 % @ 10 µg/L; 95 - 105 % @ 100 µg/L.
2. Cr(VI) precision: RSD ≤ 15 % @ 10 µg/L; ≤10 % @ 100 µg/L.
3. Cr(III) discrimination: ≤ 5% recovery @ 100 µg/L Cr(III).

4. Mean Blank Value: ≤ 1 µg/L.
5. Storage Test: $\leq 10\%$ difference between means for 0 and 24 hr samples.

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

Date Prepared: 09/16/87

Date revised: 09/03/97

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

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.

Prepared by:

_____

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Approved by:

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Ed Little Date
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Date Prepared: 6/19/87

Date Revised:09/19/97

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

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 (phenol-phthalein 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 ECRC 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. ECRC 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 $CaCO_3$ 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 ECRC SOP B4.56 for pH meter and electrode maintenance and storage.

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Linda Sappington Date
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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 $\pm 5°C$. 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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Quality Assurance Officer

Attachment A

Hatching of Brine Shrimp Eggs

1. Put approx. 3000 mls. of H_2O 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 H_2O.

3. SHAKE WELL!

4. Place bottle in a water bath at 30°F and using a airstone, moderately bubble air into the H_2O for 48 hrs.

5. After 48 hrs. pour the H_2O + 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	KMnO4	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[R])	0.25 AI	

Date Prepared: 09/18/87

Date Revised: 09/03/97

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

TEMPERATURE MONITORING OF FREEZERS AND REFRIGERATORS AT ECRC

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.

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_____

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