

# IDAHO MINING ASSOCIATION

## SELENIUM COMMITTEE

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### 1999 Interim Investigation Data Report

Southeast Idaho  
Phosphate Resource Area  
Selenium Project

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October 2000

*Prepared by:*



**MONTGOMERY WATSON**

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## **Section 1**

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# 1.0 INTRODUCTION

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This report presents stream water quality data, cutthroat trout egg viability and genetics data collected by the Idaho Mining Association (IMA) Selenium Committee as part of the 1999 Interim Investigation. Preliminary information is also presented on the avian egg study and cutthroat trout selenium feeding study. Elk and beef tissue data, which were collected as part of the Selenium Committee's 1999-2000 investigative efforts, are also included. The elk and beef data are being presented in this report because of the importance and time-critical nature of these data.

The IMA Selenium Committee initiated the Southeast Idaho Phosphate Resource Area (Resource Area) Selenium Project investigation during the fall of 1997. Initial efforts included the collection and analyses of select surface water and overburden dump vegetation samples. In 1998, comprehensive sampling and analysis of surface water, sediment, groundwater, and overburden dump soil and vegetation were conducted. Evaluations of the 1998 data indicated that additional data collection efforts were warranted. In particular, aquatic and terrestrial ecological information was needed to support refinement of preliminary human and ecological health risk assessments (Montgomery Watson [MW], 1999a).

The Selenium Committee intended to initiate aquatic and terrestrial biota sampling and analysis activities, along with continued surface water and sediment investigations, in May 1999. Time constraints in early 1999 prevented the development of a comprehensive work plan that described planned 1999 sampling and analysis activities in time to properly implement the work during the spring of 1999. However, the Selenium Committee believed it was important to start several time-critical activities including an avian egg study and cutthroat trout studies in the spring of 1999. In addition, several Interagency/Phosphate Industry Selenium Working Group (SeWG) participants indicated it was undesirable to completely forego stream water column sampling and analysis in May 1999. Consequently, the Selenium Committee proposed an interim effort to assure essential data collection activities were conducted during the spring of 1999.

The SeWG agreed that the interim investigation approach was warranted to assure that time-critical data needs were satisfied to avoid unnecessary delay in the overall Southeast Idaho Phosphate Resource Area Selenium Project (Selenium Project). In addition to providing a mechanism for collecting time-critical data, the interim approach was implemented to provide the SeWG more time to evaluate 1998 data, and to more effectively participate in planning the 1999-2000 regional investigation activities. The scope of the interim investigation was agreed to at the SeWG meeting of May 6 and 7, 1999.

## 1.1 REPORT ORGANIZATION

The contents of this report are as follows:

- Section 1.0. Introduction – This section describes the purpose of the report and the scope-of-work for the 1999 interim investigation.
- Section 2.0. Background – Section 2.0 provides project background, introduces members of the IMA Selenium Committee and the Interagency/Phosphate Industry Selenium Working Group.
- Section 3.0. Methodology – This section describes procedures and methodologies used for data collection, laboratory analysis, and data evaluation.

- Section 4.0. Data – This section presents 1999 interim investigation sampling and analysis results, as well as the results from the elk investigation and the post-mortem portion of cattle investigation conducted as part of the 1999-2000 investigation.
- Section 5.0. References – This section lists the references cited.

Supporting documentation is included in the appendices.

## 1.2 INTERIM INVESTIGATION OBJECTIVES AND SCOPE

The 1999 interim investigation included initiating several studies to avoid un-necessary delay in the Selenium Project. Select stream water column data were also generated to assure continuity in the surface water characterization efforts. The Selenium Committee presented the 1999 interim investigation objectives and scope in the *Interim Field Sampling Plan, 1999 Interim Regional Investigation/Management Study* (Interim Sampling Plan; MW, 1999b). The Selenium Project study area is shown on Drawing 1-1, *Selenium Project Study Area*.

### 1.2.1 Interim Investigation Objectives

The objectives of the 1999 Interim Investigation were:

- To characterize selenium and cadmium concentrations in surface water at select stream locations. These data will support the evaluation and implementation of best management practice alternatives developed for mitigating environmental threats.
- To characterize selenium and cadmium concentrations in bird eggs.
- To characterize the sensitivity of native cutthroat trout to elevated concentrations of selenium.

During 1998 regional investigation planning efforts, six target trace elements were identified through a preliminary, risk-based screening process (MW, 1998a). The six targeted trace elements were cadmium, manganese, nickel, selenium, vanadium, and zinc. Selenium is the only target element known to have caused an environmental problem. Field investigations in 1997 and 1998 indicate that selenium and possibly cadmium are the targeted trace elements that appear elevated near regulatory levels (MW, 1998b; 1999c). The other four targeted trace elements have been measured at, or slightly above, background concentrations. However, the measured concentrations are well below regulatory standards and have not caused any known environmental problems. Consequently, the 1999 interim investigation studies focused on characterizing selenium and cadmium concentrations.

Data collected during the 1999 interim investigation will be used to develop a better understanding of potential environmental threats associated with phosphate mining activities. The potential environmental impacts are being characterized through the impact and risk assessment processes. The Selenium Committee has conducted a preliminary risk assessment (MW, 1999c). The interim investigation data will be used to refine and revise the preliminary risk assessment. However, the next iteration of the risk assessment process is beyond the scope of this document and will be presented at a later date. Initial screening assessments are included to help put the beef and elk data into perspective.

# LEGEND

- CONTOURS
- CREEKS/RIVERS
- LAKES
- MARSH
- ROADS
- RAILROAD
- STATE LINE
- COUNTY LINE

- NATIONAL FOREST
- BUREAU OF LAND MANAGEMENT
- STATE OF IDAHO
- FORT HALL INDIAN RESERVATION

- ASTARIS PRODUCTION LLC
- FMC CORPORATION AND J.R. SIMPLOT COMPANY
- J.R. SIMPLOT COMPANY
- NU-WEST MINING, INC. OR NU-WEST INDUSTRIES, INC.
- MONSANTO COMPANY
- RHODIA INC.

- ① DRY VALLEY MINE
- ② GAY MINE
- ③ SMOKY CANYON MINE
- ④ LANES CREEK MINE
- ⑤ CONDA MINE
- ⑥ RASMUSSEN RIDGE MINE
- ⑦ MOUNTAIN FUEL MINE
- ⑧ CHAMP MINE
- ⑨ NORTH MAYBE MINE
- ⑩ GEORGETOWN CANYON MINE
- ⑪ ENOCH VALLEY MINE
- ⑫ HENRY MINE
- ⑬ BALLARD MINE
- ⑭ WOOLEY VALLEY MINE

STATE OF IDAHO

STUDY AREA

MAP KEY

SCALE: 12,000' 0 12,000' 24,000'

CONTOUR INTERVAL: NA

REV. NO.	REVISIONS	DATE	DESIGN BY	DRAWN BY	REVIEWED AND SIGNED BY
0	Issued for Draft	2/19/01	J. Wehrman	J. Gates	J. Wehrman

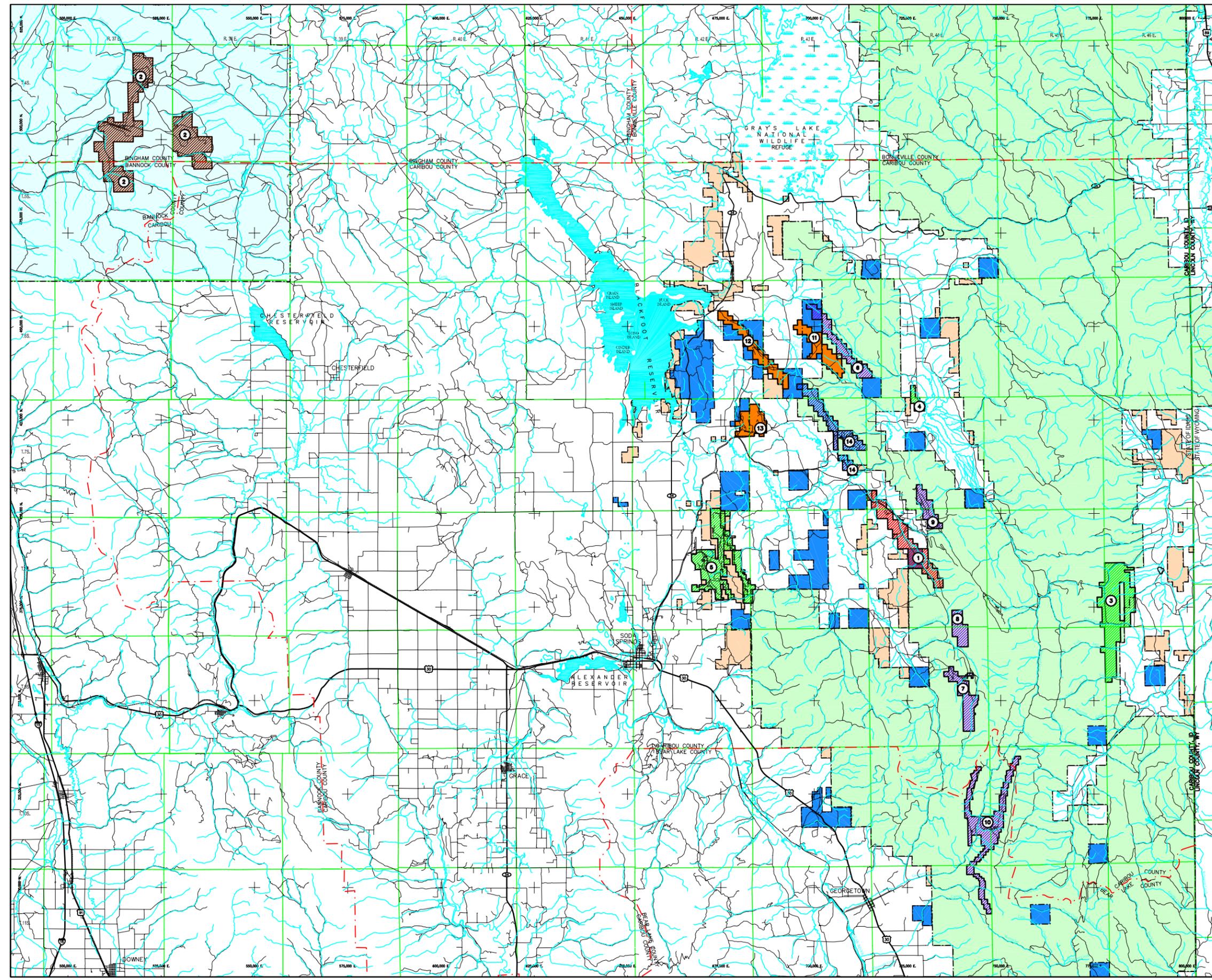
**IDAHO MINING ASSOCIATION  
SELENIUM COMMITTEE**

PROJECT: DATA REPORT FOR SURFACE WATER, SEDIMENT AND AQUATIC BIOTA SAMPLING ACTIVITIES, SEPTEMBER 1999 - APRIL 2000

DRAWING TITLE: **SELENIUM PROJECT STUDY AREA**

MONTGOMERY WATSON

Sheet 1 Of 1 Sheets  
SCALE: As Shown  
DRAWING No. 1-1



Another component of the Selenium Project is the Management Study. The management study, as defined in IMA's 1998 Sampling and Analysis Plan (Montgomery Watson, 1998a), is to be functionally equivalent to a CERCLA feasibility study. As the management study evolved, the Best Management Practice (BMP) Technical Subcommittee determined that there were three primary objectives:

1. To identify existing BMPs (whether specifically implemented to control selenium releases or not) that are currently (or historically) being used that may help reduce or eliminate selenium releases at the source of generation.
2. To communicate to interested parties, the BMPs (which are believed to reduce or eliminate selenium releases) that are currently being employed at active mine sites.
3. To develop a guidance document(s) containing a list of known BMPs (which are believed to reduce or eliminate selenium releases) which could be applied on a site-specific basis in an attempt to control selenium releases and/or threats at the source of generation.

Toward these objectives, the BMP Technical Subcommittee developed a document to communicate the BMPs currently being used at active mine sites (MW, 2000). Two other manuals will be used as operational guidance manuals to identify and select site-specific BMPs to be applied singularly, or in combination, to help reduce selenium and trace element impacts to the environment. The BMP guidance manuals are being developed independently of this document and will be presented at a later date.

### **1.2.2 Interim Investigation Scope of Work**

The data collection, analysis and evaluation activities identified as time-critical by the IMA Selenium Committee were:

- The continuation of surface water monitoring at select locations in the upper Blackfoot River watershed;
- The implementation of a two-year avian egg quality study;
- The implementation of cutthroat trout feeding, egg viability, and genetic studies;
- The compilation of municipal groundwater compliance monitoring data collected by the municipalities of Soda Springs and Fort Hall;
- The identification and mapping of mine facilities at the 14 mines in the study area;
- The development and preparation of the 1999-2000 Sampling and Analysis Plan; and,
- The continuation of the Management Study.

This report presents data collected under the tasks identified in the first four bullets. The Selenium Committee is developing maps that show the locations of all phosphate mine facilities. The maps will be presented independently of this report. The Selenium Committee completed the 1999-2000 Sampling and Analysis Plan (MW, 1999d) in August 1999, and distributed the work plan to the SeWG. 1999-2000 investigation data collection activities were initiated in September 1999 and completed in May 2000. As indicated above, several Management Study documents are being prepared under separate cover.

### 1.3 1999-2000 INVESTIGATION TIME-CRITICAL HUMAN HEALTH ISSUES

In the process of finalizing the 1997 investigation report, State of Idaho political leaders asked if selenium releases associated with phosphate mining activities could threaten human health, specifically in relation to the consumption of fish caught downstream of mines. As a result of this concern, the Selenium Committee conducted an initial trout fillet quality study as part of the 1998 regional investigation. During the 1999-2000 investigation planning process, regulatory agencies extended this concern to the consumption of wildlife and livestock that graze on reclaimed overburden dumps. Consequently, the scope of work for the 1999-2000 investigation included studies of beef and elk tissue quality (MW, 1999d).

The elk study is a cooperative effort between the Selenium Committee and Idaho Department of Fish and Game (IDFG). IDFG collected elk skeletal muscle and liver tissues during the fall of 1999 from hunters who harvested elk from two game management units that overlap the central and eastern portions of the Resource Area. The Selenium Committee had the tissues analyzed for selenium and cadmium, the analytical results validated, and with IDFG's and the Idaho Department of Health and Welfare's (IDHW) assistance, evaluated the data, which are presented in this report.

The Selenium Committee conducted a beef tissue study that investigated selenium depuration to reduce uncertainties in exposure point concentrations of selenium so that the preliminary human health risk assessment can be refined. In June 1999, Monsanto Company, one of the six member companies of the Selenium Committee, initiated that year's portion of a long-term grazing study at Henry Mine, one of the 14 mines included in the Selenium Project study area. Forty-five steers were pastured on reclaimed overburden dumps for nine weeks. To determine the rate at which selenium accumulated during this time is depurated, or removed, from edible tissue, and to better quantify trace element concentrations at the time of slaughter, 15 of the steers, along with five control steers, were randomly selected for the depuration study. Approximately one month after being removed from seleniferous pasture, the 15 treatment steers and five control steers were shipped to the University of Idaho in Moscow, where they were handled under simulated feedlot conditions for approximately four months. During the course of the study blood, serum, muscle biopsy, and liver biopsy samples were taken periodically to allow for an assessment of depuration rates. The steers were slaughtered in February 2000, and the post-mortem data for skeletal muscle, liver, heart, and kidney are presented in this report, along with a preliminary report that was submitted to the U.S. Department of Agriculture's Food Safety Inspection Service (FSIS).

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## **Section 2**

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## 2.0 PROJECT BACKGROUND

In late 1996, six horses pastured downstream of a historic phosphate mine were diagnosed with chronic selenosis. This event prompted concern by the public, local, state and federal agencies, and mine operators about potential selenium impacts to the environment. The Selenium Committee, a voluntary, ad hoc committee of the IMA, was formed in early spring 1997. The Selenium Committee was formed to identify the source and extent of selenium and other trace element impacts associated with phosphate mining activities. In the summer of 1997, additional horses pastured on a second phosphate mine were also diagnosed with selenosis. This second event increased the emphasis on characterizing the extent of possible selenium and trace element exposures within the Resource Area.

The IMA Selenium Committee consists of the following six companies currently mining or who have recently mined phosphate ore in Southeast Idaho:

- Astaris Production LLC (Astaris; a joint venture between FMC and Solutia Inc.);
- FMC Corporation (FMC);
- J.R. Simplot Company (Simplot);
- Nu-West Industries, Inc. and Nu-West Mining, Inc. (Nu-West);
- Monsanto Company (Monsanto); and,
- Rhodia, Inc. (Rhodia).

These companies and their respective active and inactive mines are identified in Table 2.1, *Phosphate Mines in the Southeast Idaho Phosphate Resource Area*. These companies or their predecessors owned, leased or operated the four active and ten inactive mines included in the Selenium Project. The locations of these mines are shown on Drawing 1-1.

<b>TABLE 2.1 PHOSPHATE MINES IN THE SOUTHEAST IDAHO PHOSPHATE RESOURCE AREA</b>		
<b>Company</b>	<b>Mines</b>	
	<b>Active</b>	<b>Inactive</b>
Astaris	Dry Valley Mine	
FMC		Gay Mine <sup>1</sup>
Simplot	Smoky Canyon Mine	Lanes Creek Mine Conda Mine Gay Mine <sup>1</sup>
Nu-West	Rasmussen Ridge Mine	Mountain Fuel Mine Champ Mine North Maybe Canyon Mine South Maybe Canyon Mine <sup>2</sup> Georgetown Canyon Mine
Monsanto	Enoch Valley Mine	Henry Mine Ballard Mine
Rhodia	None	Wooley Valley Mine
<b>Notes:</b> <sup>1</sup> Responsibility for Gay Mine is shared between the FMC Corporation and J. R. Simplot Company. <sup>2</sup> South Maybe Canyon Mine is not included in the scope of the Selenium Project. It is being addressed separately under a consent order between Nu-West and U.S. Forest Service.		

The Selenium Project, which is funded by the Selenium Committee, is being conducted with the assistance and participation of the SeWG. The SeWG is comprised of the Selenium Committee member companies, the Shoshone-Bannock Tribes, and the following federal, state, and local agencies.

- United States Forest Service (USFS)
- United States Bureau of Land Management (BLM)
- Idaho Department of Environmental Quality (IDEQ)
- Idaho Department of Lands (IDL)
- Idaho Department of Fish and Game (IDFG)
- United States Bureau of Indian Affairs (BIA)
- United States Fish and Wildlife Service (USFWS)
- United States Environmental Protection Agency (EPA)
- United States Geological Survey (USGS)
- Idaho Department of Health and Welfare (IDHW)
- Idaho Department of Agriculture
- Southeastern District Health Department

In addition to the member companies, the Shoshone-Bannock Tribes, and agencies, the Greater Yellowstone Coalition, a regional environmental organization, and numerous interested local residents participate in the Selenium Project process.

The Selenium Committee retained technical and communications consultants to assist in fulfilling its mission. Montgomery Watson (MW), an environmental technology firm, was hired in April 1997 to assist in planning and implementation of various investigations and engineering evaluations. MW has contracted with technical experts in the areas of selenium biogeochemistry (Dr. Greg Moller, veterinary toxicology (Dr. Patricia Talcott), aquatic ecology (Dr. Michael Falter), fish nutrition (Dr. Ron Hardy), rangeland ecology (Dr. Jim Kingery), ornithology (Dr. John Ratti), and population ecology (Dr. Oz Garton) from the University of Idaho. The University of Idaho and the University of California at Davis are providing state-of-the-art analytical laboratory services. The Selenium Committee has also retained local technical communications experts in the areas of agricultural science and veterinary medicine (Mr. Ed Duran and Dr. Scott MacGregor) to assist in the preparation of public education materials and in the organization of public education events. The SeWG agencies are actively involved in the Selenium Project and are providing planning and technical oversight, as well as, conducting independent studies.

The Selenium Project is a phased characterization of selenium and trace element sources and impacts within and around phosphate mining facilities. The regional investigation has the following primary objectives:

- To characterize the extent and magnitude of releases of selenium and other target trace elements (cadmium, manganese, nickel, vanadium, and zinc) from phosphate mine overburden in a broad range of environmental media, including surface water, sediment, groundwater, surface soil, and select terrestrial and aquatic biota.
- To characterize the threat of overburden chemical constituent impacts, including selenium and other target trace elements, to human and ecological health.
- To collect data that can be used to establish environmentally acceptable levels of selenium and other target elements for the purpose of developing region- or site-specific mitigation goals to protect the environment.

In July 2000, the Shoshone-Bannock Tribes and participating state and federal agencies initiated a limited interagency-driven investigation that is similar to the Selenium Committee's Selenium Project. A Memorandum of Understanding (MOU) was signed that identified IDEQ as the lead agency in the interagency-driven investigation. This interagency-driven investigation is designed to establish remedial action objectives, remediation goals, and risk-based cleanup levels for selenium and other trace elements that will be protective of human health and the environment. In addition, it will provide information to support future agency-approved site investigations and remedial actions at phosphate mines in the Resource Area. At the time of this report the various signatories to the MOU and the Selenium Committee member companies were negotiating an Administrative Order on Consent which will describe the objectives and scope-of-work for this interagency-driven investigation.

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## **Section 3**

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## 3.0 FIELD AND LABORATORY METHODOLOGIES

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This section describes sample collection and analysis methods and procedures for the municipal water-supply, surface water, and biota investigations performed during the interim 1999 regional investigation or, as is the case for the elk and beef investigations, performed during the early portion of 1999-2000 regional investigation. In addition, the data validation procedures and statistical analysis methods used are described.

### 3.1 GROUNDWATER INVESTIGATION

The SeWG requested that the IMA Selenium Committee evaluate selenium and cadmium concentrations in Soda Springs and Fort Hall, Idaho municipal water-supply systems. These water-supply systems are sampled by the municipalities to evaluate compliance with Safe Drinking Water Act (SDWA) standards.

Soda Springs has two water supply sources:

- Formation Spring (headwaters of Formation Creek); and,
- Ledger Spring (near the headwaters of Ledger Creek).

Discussions with Shoshone-Bannock Tribes representatives indicated that there are four water-supply wells for Fort Hall:

- HIS Community Well;
- Sho-Ban School Well;
- Fort Hall Town Site Well; and,
- Bannock Peak Store Well.

The City of Soda Springs Water-Wastewater Department provided the SDWA compliance monitoring data that has been collected by Soda Springs. The SDWA compliance monitoring data for the Fort Hall water-supply system was provided by the Fort Hall Water & Sewer District. In the case of the Bannock Peak Store Well, the data were obtained from the owner of the store.

### 3.2 SURFACE WATER INVESTIGATION

The SeWG determined it was important to monitor a few select stream locations during high-flow conditions in the spring 1999 to assure continuity between the 1998 and the 1999-2000 regional investigations and to fill some critical data gaps. Ten stream stations were sampled in May 1999:

- ST233 Blackfoot River downstream of the Blackfoot Reservoir
- ST232 Blackfoot River upstream of the Blackfoot Reservoir, at Blackfoot River Park
- ST019 Blackfoot River downstream of Ballard Creek, immediately upstream of railroad bridge at abandoned USGS gage
- ST020 Blackfoot River downstream of State Land Creek
- ST022 Blackfoot River downstream of Wooley Valley Creek, immediately upstream of North Trail Creek Road bridge
- ST023 Blackfoot River downstream of Dry Valley Creek
- ST113 Dry Valley Creek upstream of Blackfoot River
- ST024 Blackfoot River upstream of Dry Valley Creek

- ST026 Blackfoot River upstream of Wooley Valley Range Creek, at downstream mouth of “the Narrows”
- ST229 Blackfoot River downstream of Spring Creek
- ST145 Spring Creek upstream of Blackfoot River
- ST029 Blackfoot River upstream of Spring Creek

The stations sampled during the May 1999 monitoring event will generate data that will be used to quantify annual variability in water quality. To characterize seasonal variability and to determine the duration of the spring runoff selenium pulse, two stations (ST232 and ST113) were selected for monthly sampling from May through August. The monthly sampling of these two stations was continued in September under the auspices of the 1999-2000 regional investigation.

Stream sampling was conducted following procedures presented in the project’s 1999 Interim Sampling Plan (MW, 1999b) and 1998 Sampling and Analysis Plan (1998 SAP; MW, 1998b). Field measurements were recorded for the following parameters:

- pH;
- specific conductivity;
- temperature;
- dissolved oxygen;
- turbidity;
- oxidation-reduction potential; and,
- flow.

Water-column samples were analyzed for the parameters presented in Table 3.1, *Surface Water Laboratory Analyses*.

TABLE 3.1 SURFACE WATER LABORATORY ANALYSES			
Parameter	Method <sup>1</sup>	Laboratory Method Detection Limit	Reporting Units
<b>Target Elements</b>			
Selenium	Hydride vapor, ICP <sup>2</sup>	0.0007	mg/L
Cadmium	200.7, ICP	0.002	mg/L
<b>Other Analyses</b>			
Alkalinity	310.1	3.0	mg/L as CaCO <sub>3</sub>
Bicarbonate	Calculation	na	mg/L
Calcium	200.7, ICP	0.01	mg/L
Carbonate	Calculation	na	mg/L
Chloride	300.0, ion chromatography	0.03	mg/L
Hardness	Calculation	na	mg/L as CaCO <sub>3</sub>
Iron	200.7, ICP	0.005	mg/L
Magnesium	200.7, ICP	0.001	mg/L
Potassium	200.7, ICP	0.8	mg/L
Sodium	200.7, ICP	0.2	mg/L
Sulfate	300.0, ion chromatography	0.1	mg/L
<b>Notes:</b> <sup>1</sup> Standard EPA methods for inorganic constituent analyses with the exception of selenium. <sup>2</sup> U of I, 1996. ICP – inductively coupled plasma spectrophotometry. na – not applicable. mg/L – milligrams per liter.			

Each sample collected for selenium analysis was unfiltered and acidified to a pH < 2 with ultra-pure nitric acid. Another aliquot at each station, filtered in the field with a 0.45 µm disposable filter and acidified to a pH < 2, was submitted to the laboratory for the analysis of cadmium and cations. A third aliquot, unfiltered and un-acidified, was submitted to the laboratory for anion analysis. All samples were stored on ice or in a refrigerator and shipped on ice to the laboratories by overnight courier under standard chain of custody.

Quality assurance/quality control (QA/QC) suites were collected at a minimum rate of 10 percent. Two QA/QC suites were collected in May, and one QA/QC suite was collected during each of the monthly monitoring events. A QA/QC suite consisted of four replicate samples and an equipment rinsate. One replicate sample was analyzed by the Veterinary Diagnostic Laboratory at the University of California at Davis. The other samples were analyzed by the Analytical Sciences Laboratory (ASL) at the University of Idaho (U of I).

### **3.3 BIOTA INVESTIGATION**

The four biota investigations that are included in this report are:

- Bird egg investigation;
- Cutthroat trout investigation;
- Elk tissue investigation; and,
- Cattle investigation.

#### **3.3.1 Bird Egg Investigation**

The preliminary ecological risk assessment indicated that selenium might pose a threat to the health of certain bird species (MW, 1999a). To help refine the ecological risk assessment, the IMA Selenium Committee initiated a bird egg study to determine if phosphate mining resulted in elevated exposures to selenium or cadmium in avian populations. Dr. John Ratti, an ornithologist at the University of Idaho, was retained to conduct the study. This is a two-year study for which field activities were initiated in May 1999. Section 4 of this report includes a general summary of the first year's results. Dr. Ratti will prepare a completion report describing the entire study under separate cover.

The study design and sample collection procedures for the bird egg study are presented in Appendix A. This information was initially provided with the 1999 Interim Sampling Plan (MW, 1999b), but was incomplete. Thus, it is provided in its entirety here. The general objective of the bird egg study is to assess target trace element concentrations in eggs across various trophic groups. The data will be used to determine whether phosphate mining is responsible for increased concentrations in eggs and, if so, to what degree. The information will be used to refine the preliminary ecological health risk assessment to estimate the threat posed by the degree of exposure.

Four distinct habitat strata are being sampled for both phosphate mining and control areas:

- 1) tributary streams (small, generally 1<sup>st</sup>- or 2<sup>nd</sup>-order streams);
- 2) ponds and wetlands;
- 3) rivers; and,
- 4) lakes and reservoirs.

Prior to egg collection, positive identification to species is made in the field by observing a bird at the nest. One egg was randomly selected from each nest using a systematic rotational sequence. After an egg was collected it was immediately placed into an iced cooler. At the end of each day the eggs were transferred to a secured refrigerator. Samples were transported to the U of I's ASL by overnight carrier under standard chain of custody.

At the laboratory the eggs were analyzed for reproductive effects following analytical procedures developed by the ASL (U of I, 1999). The protocols for assessing reproductive effects were developed in consultation with USFWS researchers and are included in Appendix A. A summary of the protocol is as follows:

- Eggs were stored in the laboratory at 4°C until analyzed.
- The egg morphometry was determined by measuring length, width, weight, and volume. Volume was determined by displacement in water if the shell was not cracked. If the shell was cracked, the volume was determined by a species-specific calculation.
- Data were then collected for reproductive-effects assessment. Each egg was evaluated to see if the embryo was in the correct position and then emptied into a clean, sterilized Petri dish. Each egg was photographed for later evaluation.
- The content of each egg was then homogenized and an aliquot was analyzed for targeted trace element concentrations. A second aliquot was analyzed for moisture content.

Standard laboratory methods were used to measure egg selenium and cadmium concentrations and determine moisture content. Table 3.2, *Bird Egg Investigation Laboratory Analyses*, presents the laboratory analytical methods.

<b>TABLE 3.2 BIRD EGG INVESTIGATION LABORATORY ANALYSES</b>			
<b>Parameter</b>	<b>Method</b>	<b>Laboratory Method Detection Limit</b>	<b>Reporting Units</b>
Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
Cadmium	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.02	mg/kg (wet)
Moisture content	Gravimetric, ASL method <sup>3</sup>	na	Percent
<b>Notes:</b> <sup>1</sup> U of I, 1997a. <sup>2</sup> U of I, 1997b. <sup>3</sup> U of I, 1997c. ICP – inductively coupled plasma spectrophotometry. mg/kg (wet) – milligrams per kilogram on a wet-weight basis. na - not applicable.			

### 3.3.2 Elk Investigation

The SeWG proposed in August 1999 that an elk tissue quality investigation be conducted within the Resource Area. A cooperative study between the Selenium Committee and the IDFG was undertaken to determine the levels of selenium and cadmium in liver and skeletal muscle. The purpose of the study was to determine if levels of these targeted trace elements were elevated as a result of increased exposures related to phosphate mining and, if so, to quantify any threat posed to human health. The information would also be used, to the extent possible, to evaluate any threat to the health of the elk themselves.

In September 1999, the IDFG sent a letter to hunters holding permits in Game Management Units 76 and 66A to inform them of the sampling effort and request assistance. These two game management units overlap the central and eastern districts of the Resource Area. The letter indicated that muscle samples would be collected from harvested elk as they came through IDFG check stations, and it asked hunters to take a liver sample when dressing out the carcass, which would then be collected by IDFG at either a check station or a designated drop-off location. A copy of the letter is included in

Appendix C. The IDFG forwarded the samples collected to the U of I's ASL, and the Selenium Committee coordinated the analysis of the samples.

Information recorded for each elk included tissue(s) sampled, hunter's hunting license number, age of the elk, sex of the elk, kill date, and kill-site location. A copy of the data sheet is also included in Appendix C. IDFG collected samples and information at two check stations on October 26, 27, 30, and 31, and on November 6 and 7. The locations of the check stations were:

- Idaho State Highway 34 just north of Soda Springs; and,
- Lower Georgetown Canyon Road near Georgetown.

The alternative sample drop-off locations were:

- IDFG's Pocatello office;
- USFS's Soda Springs office; and,
- USFS's Montpelier office.

Upon receipt by IDFG or USFS personnel, the samples were placed in an iced cooler. At the end of each day the coolers were transported to IDFG's office in Pocatello where the samples were stored in a secured freezer. Samples were then shipped to the ASL by overnight carrier under standard chain of custody.

The elk samples were analyzed using the methodologies presented in Table 3.3, 1999 *Elk Investigation Laboratory Analyses*. Because the focus of the 1999 interim investigation was narrowed to selenium and cadmium, the Selenium Committee only validated and evaluated those two targeted trace elements. A brief summary of a U of I veterinary toxicologist's perspective on the remaining trace elements is presented in Section 4.

TABLE 3.3 1999 ELK INVESTIGATION LABORATORY ANALYSES			
Parameter	Method	Laboratory Method Detection Limit	Reporting Units
Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
Cadmium	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.02	mg/kg (wet)
Copper	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.03	mg/kg (wet)
Iron	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.04	mg/kg (wet)
Lead	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.23	mg/kg (wet)
Manganese	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.01	mg/kg (wet)
Molybdenum	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.09	mg/kg (wet)
Zinc	Heavy metal screen by ICP for tissue analysis <sup>2</sup>	0.01	mg/kg (wet)
Moisture content	Gravimetric, ASL method <sup>3</sup>	na	Percent
<b>Notes:</b> <sup>1</sup> U of I, 1997a. <sup>2</sup> U of I, 1997b. <sup>3</sup> U of I, 1997c. ICP – inductively coupled plasma spectrophotometry. mg/kg (wet) —milligrams per kilogram on a wet-weight basis. na —not applicable.			

### 3.3.3 Cattle Investigation

To provide quantitative selenium data that could be used to refine the human health risk assessment the Selenium Committee expanded upon an existing cattle grazing study that was being conducted on reclaimed overburden dumps at Henry Mine by Monsanto, IDL, and U of I. The grazing study was a multiple-year endeavor that was initiated several years before selenium became an issue of concern. During the past three years of the grazing study, the scope was expanded to include characterization of selenium levels in dump surface soil and vegetation, and in yearly steer blood and serum.

The grazing study was conducted during a 9-week period each summer, a common grazing rotation for montane pastures in and near the Caribou National Forest. Three separate, fenced pastures were grazed by three groups of yearling steers. (This is not a representative exposure scenario for montane-grazed steers having access to reclaimed overburden dumps. In a typical scenario, a grazing permittee would not confine grazing animals to just reclaimed dumps, the animals would graze the entire range which would include both native, undisturbed pasture and revegetated pasture on dumps.) During the summer of 1999, blood and serum samples were obtained just before the steers were exposed to the reclaimed pasture, at three-week intervals while on the pasture, and immediately upon removal from the Henry Mine pasture.

Because of the magnitude of uncertainties associated with the beef ingestion component of the Selenium Committee's preliminary human health risk assessment (MW, 1999b), the Selenium Committee purchased 20 steers to conduct a selenium depuration study. Fifteen of the steers were animals used in the grazing study, with five randomly selected from each of the three reclaimed, seleniferous pastures. Another five steers were randomly selected from a native, undisturbed, non-seleniferous pasture. This investigation was conducted under the direction of Dr. Jim Kingery, a rangeland ecologist at the U of I, who was also the principal investigator for the grazing study. The depuration investigation was conducted under conditions that simulated the normal handling of steers as they are prepared for market. The steers were pastured for one-month on lowland (non-seleniferous) pasture after removal from the Henry Mine pastures followed by four months in a feedlot consuming a diet containing selenium in a concentration of 0.3 mg/kg (wet, but air-dried; the maximum concentration of selenium allowed under feedlot regulations).

The primary objectives of the Selenium Committee's beef depuration study were:

- To determine whether selenium levels in beef tissues from steers exposed, under relatively worst-case conditions, to pasture affected by phosphate mining are elevated and, if so, to what degree;
- To characterize levels of selenium in skeletal muscle and other soft tissues (liver, heart, and kidney) to reduce uncertainties estimated in the preliminary human health risk assessment; and,
- To estimate the rate of depuration (removal) of selenium from beef tissue to reduce uncertainties quantified in the preliminary human health risk assessment.

A secondary objective was to use information obtained to evaluate the potential for health effects to the steers themselves and, by extrapolation, to other grazing cattle and wildlife.

Planning for the depuration investigation was initiated late in the planning phase for the 1999-2000 regional investigation. As a result, the final protocols for the depuration investigation have not been distributed to the recipients of the 1999-2000 SAP. The study protocols are presented in Appendix D.

Each steer was sampled for blood and serum when they arrived at the U of I Beef Center, and then every two weeks. In addition, liver and muscle biopsy samples were collected three times, at approximately Day 37, 107 and 157 after leaving the Henry Mine pastures. Skeletal muscle, liver, heart, and kidney samples were collected from each animal at the time of slaughter. The steer tissue samples were analyzed for the parameters tabulated in Table 3.5, *Beef Depuration Study Laboratory Analyses*.

### 3.3.4 Cutthroat Trout Investigation

Native Yellowstone cutthroat trout is the highest-valued aquatic species in the upper Blackfoot River system (D. Scully, IDFG Southeast Regional Fisheries Manager, personal communication). To determine if observed selenium concentrations are harming native cutthroat trout and to assist with the development of site-specific mitigation goals, the IMA Selenium Committee initiated a two-year cutthroat trout investigation. There are three components to this investigation. One is an egg-viability study to assess if observed selenium concentrations are causing birth defects. The second is a feeding trial to assess if dietary selenium impacts growth rates, survivorship, or subsequent breeding success. The third is a genetic analysis to evaluate whether test results might be skewed by survivorship bias.

Dr. Ron Hardy, a fish nutritionist with the U of I's Aquaculture Research Institute at the Hagerman Fish Culture Experiment Station, was retained to conduct the first two aspect of the cutthroat trout investigation. The egg-viability study was undertaken as a one-time event, but the feeding trial was undertaken as a study of at least two years in duration. Dr. Matt Powell, a fish geneticist at the Hagerman Fish Culture Experiment Station, was retained to conduct the genetic analysis, which was undertaken as a one-time event. This report presents the results of the egg-viability study and genetics analysis, and provides a general summary of the first year of the feeding trial.

The study methodologies and protocols are presented in Appendix B. The laboratory chemical analytical methodologies are presented in Table 3.4, *Cutthroat Trout Investigation Laboratory Analyses*.

<b>TABLE 3.4 CUTTHROAT TROUT INVESTIGATION LABORATORY ANALYSES</b>			
<b>Parameter</b>	<b>Method</b>	<b>Laboratory Method Detection Limit</b>	<b>Reporting Units</b>
Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
Moisture content	Gravimetric, ASL method <sup>2</sup>	na	Percent
<b>Notes:</b> <sup>1</sup> U of I, 1997a. <sup>2</sup> U of I, 1997c. ICP – inductively coupled plasma spectrophotometry. mg/kg (wet) - milligrams per kilogram on a wet-weight basis. na - not applicable.			

### 3.4 QUALITY ASSURANCE, DATA VALIDATION, AND STATISTICS

Rigorous quality assurance/quality control (QA/QC) and data validation techniques were used in accordance with EPA (1994, 1995, 1996) and Association of Official Analytical Chemists (Linnig, Mandel, and Peterson, 1954; Mandel and Linnig, 1957; Wernimont, 1985) guidelines to assure that accurate results were obtained. Trace element concentrations are often present in very low concentrations. The strict adherence to the EPA data validation guidelines results in the censoring of low end of the data (i.e., concentrations lower than a specified reporting limit are normally reported as being below the detection limit [BDL]).

TABLE 3.5 BEEF DEPURATION STUDY LABORATORY ANALYSES					
Sample Type	Number of Samples	Parameter	Analytical Method	Laboratory Method Detection Limit	Reporting Units
<b>120-Day Feedlot Simulation</b>					
Liver, Skeletal muscle	60	Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
Whole blood	540	Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
Serum	540	Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
		Calcium	Trace element screen by ICP <sup>2</sup>	0.070	mg/kg (wet)
		Copper	Trace element screen by ICP <sup>2</sup>	0.070	mg/kg (wet)
		Iron	Trace element screen by ICP <sup>2</sup>	0.22	mg/kg (wet)
		Magnesium	Trace element screen by ICP <sup>2</sup>	0.040	mg/kg (wet)
		Phosphorus	Trace element screen by ICP <sup>2</sup>	2.00	mg/kg (wet)
		Zinc	Trace element screen by ICP <sup>2</sup>	0.08	mg/kg (wet)
<b>Post-Mortem</b>					
Liver, Skeletal muscle, Heart, Kidney	20	Selenium	Vapor generation, ICP <sup>1</sup>	0.005	mg/kg (wet)
		Cadmium	Heavy metal screen by ICP for tissue analysis <sup>3</sup>	0.02	mg/kg (wet)
		Moisture content	Gravimetric, ASL method <sup>4</sup>	Na	percent
<b>Notes:</b> <sup>1</sup> U of I, 1997a. <sup>2</sup> U of I, 1997d. <sup>3</sup> U of I, 1997b. <sup>4</sup> U of I, 1997c. ICP – inductively coupled plasma spectrophotometry. mg/kg (wet) — milligrams per kilogram on a wet-weight basis. na - not applicable.					

By using the Association of Official Analytical Chemists (AOAC) guidelines the Selenium Committee has avoided censoring the data, which results in negative concentrations sometimes being reported. These negative values are an artifact of sampling and analysis noise. The benefits of doing this, in addition to eliminating any noise-induced biases, include not having to estimate concentrations reported as BDL for subsequent statistical analysis, an improvement in data resolution, and an improvement in overall data quality (G. Moller, Technical Director, University of Idaho Animal Sciences Laboratory, personal communication).

Estimating BDL concentrations, especially in trace element data sets where it is common to have a majority of results reported as BDL, can impart substantial error and uncertainty in statistical analyses. What can happen is that a laboratory believes an instrument is reporting accurate values, but in actuality, there is a small breakdown of effective error trapping at values approaching a detection limit. When a data manager only relies on the laboratory performance estimate, it is quite possible that bias is being introduced to the data set.

All analytical methods have bias (G. Moller, personal communication). The bias can result in values being greater than true (positive) or less than true (negative). By using the AOAC guidelines, the resolution of the Selenium Committee's data is enhanced and quantifies sampling and analysis program performance and any biases. The Selenium Committee's laboratories report all of their results numerically. Statistical analysis of laboratory and field blanks indicates that selenium concentrations greater than approximately 0.0015 mg/L are statistically meaningful. Finally, the improvement in overall quality of the Selenium Committee's data is evidenced by a general enhanced agreement between the primary and quality assurance (QA) laboratories' data after the QA/QC adjustments are made.

Descriptions of the QA/QC procedures, data validation, and statistics are provided below.

### 3.4.1 Quality Assurance Procedures

The data were subjected to a rigorous QA/QC process. In addition to the primary samples, field and laboratory QA samples were collected and analyzed. QA samples were collected and analyzed, by environmental medium, at a minimum rate of ten percent of the total number of samples.

Surface water was the only medium with field collected QA samples. A field QA suite consisted of five samples: four replicate samples and a field equipment blank. Three replicate and the field blank samples were analyzed by the primary laboratory at the U of I. One of the replicates was spiked to allow for quantification of matrix effects noise. The fourth replicate sample was analyzed at the QA laboratory at the University of California at Davis. The QA laboratory results were compared to those of the primary laboratory using a prediction interval defined on a 5 percent experiment-wise false-positive statistical error rate (Hahn and Meeker, 1991 [for calculation of prediction intervals]; Green, 1979 [for calculation of experiment-wise error rates]). Laboratory QA/QC samples included laboratory blanks, laboratory matrix spikes, laboratory duplicates, and laboratory reference samples, which were measured in conjunction with each sample delivery group.

For the biota media (tissue samples), the primary laboratory generated QA replicates and equipment blanks in the laboratory during sample preparation. One of the replicates was sent to the QA laboratory for analysis. Replicate QA samples could not be obtained from beef tissue biopsies because of small sample size. In addition, several elk samples contained such a small quantity of tissue that these samples could not be used as QA samples. The laboratory QA samples consisted of laboratory duplicate samples and standard laboratory reference samples.

The raw laboratory data, including primary samples, QA/QC replicate samples, equipment rinsate samples, laboratory blanks, laboratory matrix spike samples, laboratory duplicate samples, and laboratory reference standard results are presented in Appendix E. These data are presented in an electronic format as received from the analytical laboratories, with the exception that spike values or expected concentrations for matrix spikes, blank spikes and laboratory control standards. A column was added to present this information.

### 3.4.2 Data Validation Procedures

Data validation was conducted consistent with EPA (1994 and 1996) and AOAC (Linnig, Mandel, and Peterson, 1954; Mandel and Linnig, 1957; Wernimont, 1985) guidance using data reported by the laboratories from the QA/QC samples. The EPA validation procedures are described in SOP-NW-18.1, *Data Validation* (MW, 1998a). The AOAC validation process, which is fundamentally a linear regression method that attempts to quantify constant bias and proportional bias, quantified field and laboratory uncertainties using four parameters for each analyte:

- mean laboratory blank concentration ( $b_L$ );
- mean laboratory standard slope ( $m_L$ );
- mean field blank concentration ( $b_F$ ); and,
- mean matrix spike slope ( $m_F$ ).

The general quality of the laboratories' results can be readily assessed by these parameters. Small values of  $b_L$  and  $b_F$  that are close to 0 are indications of high data quality, as are values of  $m_L$  and  $m_F$  close to 1.

The four data validation parameters are then applied to the analytical results to enhance the data through minor corrections. First,  $b_L$  is subtracted from each result. The effect of this adjustment is to define the mean laboratory blank as having an effective concentration of 0 and to correct for any systematic bias generated in the laboratory. Then, any laboratory bias is further accounted for and corrected by dividing the laboratory-blank-adjusted result by  $m_L$ . The overall laboratory-adjusted result,  $x_{LA}$ , is thus:

$$x_{LA} = \frac{x_L - b_L}{m_L}$$

where  $x_L$  denotes the raw result reported by the laboratory. Because the QA laboratory does not conduct field blank or matrix spike analyses, comparisons between the two laboratories are performed on the basis of laboratory-adjusted results.

Field bias, in the form of sampling, sample handling, or matrix interferences in analysis, is accounted for and corrected by applying the  $b_F$  and  $m_F$  parameters in an identical manner to generate a field-and-laboratory-adjusted result,  $x_{FLA}$ :

$$x_{FLA} = \frac{x_{LA} - b_F}{m_F}$$

If a particular type of QA sample was not available, default values of 0 and 1 were used for the  $b$  and  $m$  parameters, respectively (i.e., no adjustment is made on the basis of missing QA data, and missing data are explicitly noted). For solid matrix samples (e.g., biological tissues), both the laboratory blanks and field blanks are aqueous. However, the solid sample must be digested, the process of converting it into an aqueous matrix for analysis. During digestion the solid undergoes dilution, a process not experienced by the blanks. For purposes of comparability, the Selenium Committee applied the mean solid dilution factor experienced by the actual samples to the associated blanks to account for the dilution that would occur if it would be feasible to obtain solid blanks. The application of this solid dilution factor to blanks associated with the analysis of solid matrices avoids underestimation of blank biases, which would result in an un-representatively high estimation of blank data quality (depending, of course, upon the exact dilution factor used).

In this report the validated data are reported along with the four data validation parameters for each analyte. Validation is conducted on a set of raw data that consists of a laboratory's standard digital report (which includes censored data) that is then supplemented, for those values reported as BDL in the digital report, with the information contained on a hard copy of the laboratory's instrument readout forms. The Selenium Committee's raw data thus consists of digital files of uncensored data (which, however, preserve, for reference, the original censoring done by the laboratory in the process of generating its digital report).

To provide an idea of the magnitudes of the adjustments, selenium water and elk liver data are used here as examples. For the May 1999 surface water sampling event, the selenium data validation parameters are:

- $b_L = 0.00002000$  mg/L;
- $m_L = 1.010$ ;
- $b_F = -0.0001283$  mg/L; and,
- $m_F = 1.124$ .

Thus, if the laboratory would have reported a concentration of 0.005 mg/L (the chronic cold water biota standard), the validated result would be 0.0045 mg/L, a difference of -10 percent. If the laboratory would have reported a concentration of 1.7 mg/L (approximately the highest concentration observed during the regional investigation to date, at a overburden dump seep), the validated result would be 1.5 mg/L, a difference of -12 percent.

For the fall 1999 elk sampling event, the selenium liver data validation parameters are:

- $b_L = -0.007202$  mg/kg (wet);
- $m_L = 1.041$ ;
- $b_F = 0$  (no field blanks were analyzed); and,
- $m_F = 0.9921$ .

Thus, if the laboratory would have reported a concentration of 2.0 mg/kg (wet), the validated result would be 1.9 mg/kg (wet), a difference of -5.0 percent. If the laboratory would have reported a concentration of 13 mg/kg (wet) (the highest concentration observed), the validated result would be 13 mg/kg (wet), no difference after results are rounded and presented to two significant figures.

As can be seen from the above examples, the effects of the adjustments are generally small. They are, however, meaningful in that they incorporate the QA data in correcting for sampling and analytical biases. The American Chemical Society (ACS) contends that “quantitative interpretation, decision-making and regulatory actions should be limited to data at or above the limit of quantitation.” By not censoring the data and by actually putting QA/QC data to use, the limit of quantitation has been eliminated, making the analytical process more transparent, and documented biases are accounted for. Both of these issues have important consequences for decision-making and regulatory actions because the higher quality data resulting is more reliable and has enhanced resolution, which is especially critical given that potential selenium action levels are at or well below traditional commercial laboratory detection limits.

This data validation methodology was not applied to the steer tissue data; rather, the Dr. Kingery’s researchers validated the data using the procedures they used in the preceding grazing study. The Selenium Committee also did not apply the methodology to the non-targeted trace element data [i.e., the non-selenium and non-cadmium data] generated on the elk tissue samples.

### 3.4.3 Statistical Methods

A variety of statistical methods are used in the process of validating and examining the Selenium Project data. The methods used are presented in this subsection.

In the data validation subsection above, use of mean (or arithmetic average) blank concentrations was mentioned. The distribution of blank results is assumed to be normal (i.e., distributed on a bell-shaped curve), an assumption that is appropriate for analytical bias. Sample means and sample standard deviations from normally distributed populations of values are calculated using the AVERAGE and STDEV functions in Microsoft® Excel (Microsoft, 1997). The sample mean of a normally distributed population of values is a minimum variance estimator of the true population mean. The sample standard deviation, however, is an asymptotically estimator of the true population standard deviation; i.e., the bias in the sample standard deviation diminishes with larger sample size. To remove the bias in the sample standard deviation (i.e., to avoid underestimating the true population standard deviation), a bias correction factor, which is a function of degrees of freedom in the data set, is applied (Diem, 1962).

Environmental concentration data, along with most other environmental and biological data, are not well modeled by a normal distribution. Lognormality—i.e., where the logarithms of the data (or logarithmically transformed data) are normally distributed—is a better model for such data, and the one used by the Selenium Committee as a null hypothesis. The sample mean and sample standard deviation of log-transformed data must be back-transformed into the original units in arithmetic space. The minimum variance back-transformation documented in Gilbert (1987) is used. Simulations support the claim that the back-transformation documented by Gilbert is unbiased with respect to estimation of the arithmetic mean, but the same simulations seem to indicate that there is an underestimating bias in the back-transformation to the arithmetic standard deviation. To remove

this apparent bias, the bias correction factor (Diem, 1962) is applied and simulations seem to indicate that this works to avoid the underestimation of uncertainty.

Because the Selenium Committee's environmental concentration data are uncensored, they often contain negative concentrations as a result of the analytical noise predominating in samples with concentrations that would generally be considered as being BDL. Log-transforms (the Selenium Committee has chosen to generally use natural logarithms, denoted  $\ln$ ) can not be done on data sets containing negative data without the lower bound of the data set first being subtracted from each value. Out of convenience the lower bound of a lognormal distribution is often assumed to be zero, and subtracting zero from the validated concentration would have no effect. When analytical bias imparts negative concentrations in the data, however, the assumption of a zero lower bound is obviously no longer valid. For the Selenium Project, the lower bound is estimated to be  $-5$  times the estimated standard deviation of the blank data associated with each analyte. If such value is of insufficient magnitude to render all positive values for  $\ln$ -transformation, the lowest value in the data set is identified and the lower bound estimated to be just sufficiently lower to allow all differences to be positive.

Excel's (Microsoft, 1997) statistical functions are used to conduct tests for homogeneity of sample variances (the F-test using the FDIST or FINV functions), to obtain critical values for comparisons of means (the t-test using the TDIST or TINV functions), and to calculate standardized normal variant scores (z scores) for data distributions (using the NORMSINV function). Excel's Data Analysis tool is used to conduct single factor analysis of variance (ANOVA), calculate correlations, and conduct linear regressions.

One-sided tolerance bounds are calculated to present operative upper bounds for both blank and background data. The calculation assumes a normally distributed population of values, and thus is done on the validated blank data directly, and on the  $\ln$ -transformed background data and then back-transformed to concentration units. The method for calculating these tolerance bounds is documented in Hahn and Meeker (1991). The back-transformation of the background bounds is a matter of exponentiating (taking the anti-log) of the bound calculated in logarithmic space then adding the estimated lower bound of the data set. The tolerance bound used is the 0.95/0.05 bound—the 95<sup>th</sup> percentile of the distribution of interest (e.g., blank data or background data) defined with a false-positive error rate of 5 percent (i.e., a confidence level of 95 percent). Such bounds are conservative in nature because, at the 95<sup>th</sup> percentile, they have a built-in 5 percent failure rate. This built-in failure rate should be considered when making comparisons to these tolerance bounds.

As mentioned in the QA subsection above, prediction intervals are used to compare the results of samples split between laboratories. This procedure is also documented in Hahn and Meeker (1991). Miscellaneous statistical tests (e.g.,  $t^2$ -test for comparing two means under the assumption of unequal variances, Fisher's least significant difference [LSD] test for comparison of means following a positive ANOVA) are documented in Ott (1977).

Two multivariate procedures are used, principal components analysis (PCA) and minimum variance cluster analysis (MVCA). These procedures are carried out with Multi-Variate Statistical Package (Kovach Computing Service, 1999). PCA is an objective ordination algorithm, "a procedure for adapting a multidimensional swarm of data points in such a way that when it is projected onto a two-space (such as a sheet of paper) any intrinsic pattern the swarm may possess becomes apparent" (Pielou, 1984). PCA is thus a pattern-recognition procedure that can be used to reduce the dimensionality of a complex and redundant data set to make it easier to understand. Pielou (1984) provides a detailed explanation of PCA.

MVCA is an objective form of cluster analysis through which sampling stations can be classified into similar groups. There are many types of clustering algorithms, but MVCA is the only one that is

amenable to objective statistical analysis to determine the significance of clusters. Pielou (1984) also provides a detailed explanation of MVCA, and the statistical method used to determine whether clusters are significant or not (an F-test) is documented in Orlóci (1978).

In conducting statistical hypothesis tests of significance, a 5 percent false-positive error rate (i.e., a 95 percent level of confidence) is used by convention. This targeted error rate is denoted as “ $\alpha$ ,” and the actual error rate is denoted as “ $p$ .” If  $p < \alpha$ , the test being performed is regarded as significant and the null hypothesis is rejected. When a number of comparisons,  $c$ , are being conducted, each at  $\alpha = 0.050$ , the effective error rate, or experiment-wise error rate,  $\alpha_{\text{experimental}}$ , increases rapidly (Green, 1979):

$$\alpha_{\text{experimental}} = (1 - \alpha)^c .$$

Thus, if  $\alpha = 0.050$  and  $c = 20$ ,  $\alpha_{\text{experimental}} = 0.64$  and there is a 64 percent chance of falsely rejecting the null hypothesis. To set  $\alpha_{\text{experimental}}$ , an  $\alpha$  must be used for each comparison that satisfies the following equation:

$$\alpha = (1 - \alpha_{\text{experimental}})^{1/c} .$$

Thus, for the desired  $\alpha_{\text{experimental}} = 0.050$ ,  $\alpha = 0.0026$ . For t-tests and F-tests it is easy to calculate  $\alpha_{\text{experimental}}$  using these equations and Excel’s statistical functions. For more complex tests (e.g., the calculation of upper tolerance bounds, which uses a non-central t-distribution), Excel does not present the necessary functions and existing tables of statistical values are limited to a few values of  $\alpha$ . In these instances one must either be aware of the multiple comparison issue or consider using a lower value of  $\alpha$ , one that probably does not result in the exact  $\alpha_{\text{experimental}}$  desired.

Thus, for the desired  $\alpha_{\text{experimental}} = 0.050$ ,  $\alpha = 0.0026$ . For t-tests, F-tests, and correlations (using, for the latter, z-transformations as documented in Diem (1961) it is possible to calculate  $\alpha_{\text{experimental}}$  using these equations and Excel’s statistical functions. For more complex tests (e.g., the calculation of upper tolerance bounds, which uses a non-central t-distribution), Excel does not present the necessary functions and existing tables of statistical values are limited to a few values of  $\alpha$ . In these instances one must either be aware of the multiple comparison issue or consider using a lower value of  $\alpha$ , one that probably does not result in the exact  $\alpha_{\text{experimental}}$  desired.

Similarly, where  $p$  is calculated and presented, it is converted to  $p_{\text{experimental}}$  as follows:

$$p_{\text{experimental}} = 1 - (1 - p)^c .$$

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## **Section 4**

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## 4.0 DATA RESULTS

This section describes the results, or provides a progress report, for the municipal water-supply, surface water, and biota (bird and cutthroat trout) studies undertaken during the 1999 interim investigation. In addition, some data obtained under the 1999-2000 regional investigation, data that are currently available, are reported here—a complete set of the data collected under the elk study, and the post-mortem data collected under the cattle investigation.

### 4.1 GROUNDWATER INVESTIGATION

At the request of the SeWG, the Selenium Committee evaluated municipal water-supply selenium and cadmium data that have been collected by the municipalities of Soda Springs and Fort Hall. These data were collected by the municipalities to comply with the Safe Drinking Water Act, and were provided to the Selenium Committee by the municipalities.

#### 4.1.1 Soda Springs Compliance Monitoring Data

Soda Springs has two water-supply sources:

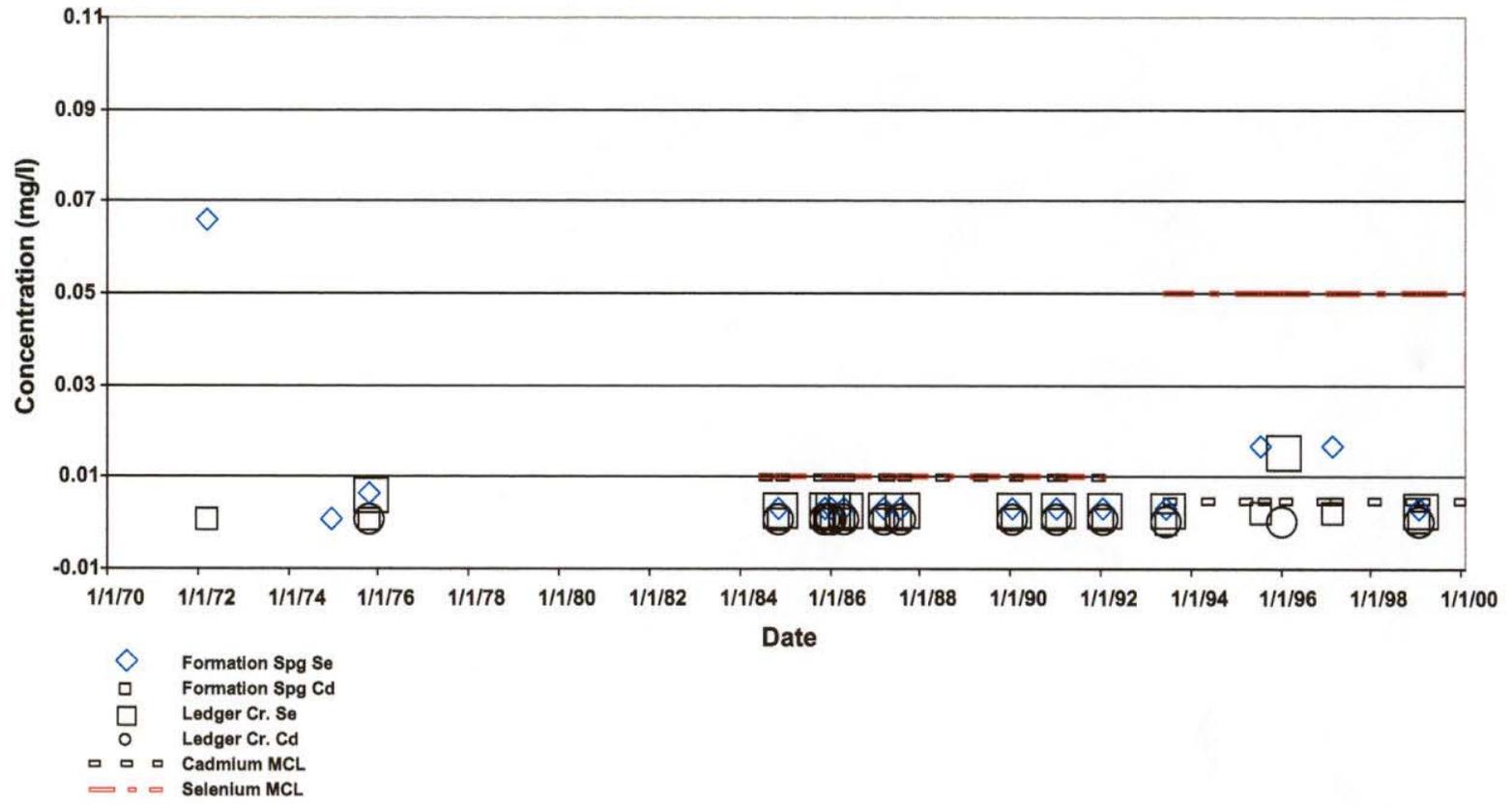
- Formation Spring; and,
- Ledger Spring.

Infrequent analyses for selenium and cadmium occurred prior to 1990. Since 1990, Soda Springs has generally analyzed one sample each year from each of the two water-supply sources with the exceptions of 1994 and 1998. The selenium and cadmium results for the Soda Springs water-supply compliance monitoring are presented in Table 4.1, *Soda Springs Water-Supply Selenium and Cadmium Data*. Drawing 4-1, *Temporal Trends of Soda Springs Water-Supply Selenium and Cadmium Concentrations*, graphically shows the data and compares it against Safe Drinking Water Act standards.

<b>TABLE 4.1 SODA SPRINGS WATER-SUPPLY SELENIUM AND CADMIUM DATA<sup>1</sup></b>						
Date	Formation Spring		Ledger Creek		Selenium MCL <sup>2</sup>	Cadmium MCL <sup>2</sup>
	Selenium	Cadmium	Selenium	Cadmium		
3/72	<0.1	< 0.001		< 0.001		
12/74	0.001	< 0.001		< 0.001		
10/75	<0.01	< 0.001	<0.01	< 0.001		
11/84	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
11/85	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
1/86	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
4/86	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
3/87	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
7/87	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
1/90	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
1/91	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
1/92	<0.005	< 0.001	<0.005	< 0.001	0.01	0.01
6/93	<0.005	<0.0001	0.005	0.0001	0.05	0.005
7/95	<0.025	<0.003			0.05	0.005
1/96			0.016	<0.0005	0.05	0.005
2/97	<0.025	<0.003			0.05	0.005
1/99	<0.025	<0.0005	<0.005	<0.0005	0.05	0.005

**Notes:** <sup>1</sup>All concentrations are reported in mg/L.  
<sup>2</sup>MCL – maximum contaminant level in mg/L; USEPA, 1986 and 1994b.  
 < – indicates the reported value was less than the detection limit.  
 A blank cell indicates that there was no reported value.

## Temporal Trends of Soda Springs Water-Supply Selenium and Cadmium Concentrations



0	For 1999 Interim Investigation Data Report	10/20/00	J. Weinman	M. Mathison	J. Weinman		
REV. No.	REVISIONS	DATE	DESIGN BY	DRAWN BY	REVIEWED AND SIGNED BY		
<b>MONTGOMERY WATSON</b>				PROJECT No. 1227028.021805 AutoCAD FILE 1999INTERIM-FIG4-3 SCALE: N/A		FIGURE No. <b>4-1</b>	

**IDAHO MINING ASSOCIATION  
 SELENIUM COMMITTEE**  
**TEMPORAL TRENDS OF SODA SPRINGS  
 WATER-SUPPLY SELENIUM AND  
 CADMIUM CONCENTRATION**

Selenium and cadmium concentrations were generally reported as being below their respective detection limits. As shown in Drawing 4-1, the reported values were also always less than their respective drinking water standards, except for the 1972 sample from Formation Spring, < 0.1 mg/L, which was reported as being below the detection limit at the time. The EPA did not have a drinking water standard for selenium prior to 1976, and the analytical procedures for selenium analysis were not as reliable as they are today.

#### 4.1.2 Fort Hall Compliance Monitoring Data

The Fort Hall Water & Sewer District provided the Selenium Committee with Fort Hall water-supply data for 1990 through 1999. We do not know if data is available before 1990. A review of compliance monitoring data shows that there were no analyses for selenium or cadmium in any of the four water-supply wells.

### 4.2 SURFACE WATER INVESTIGATION

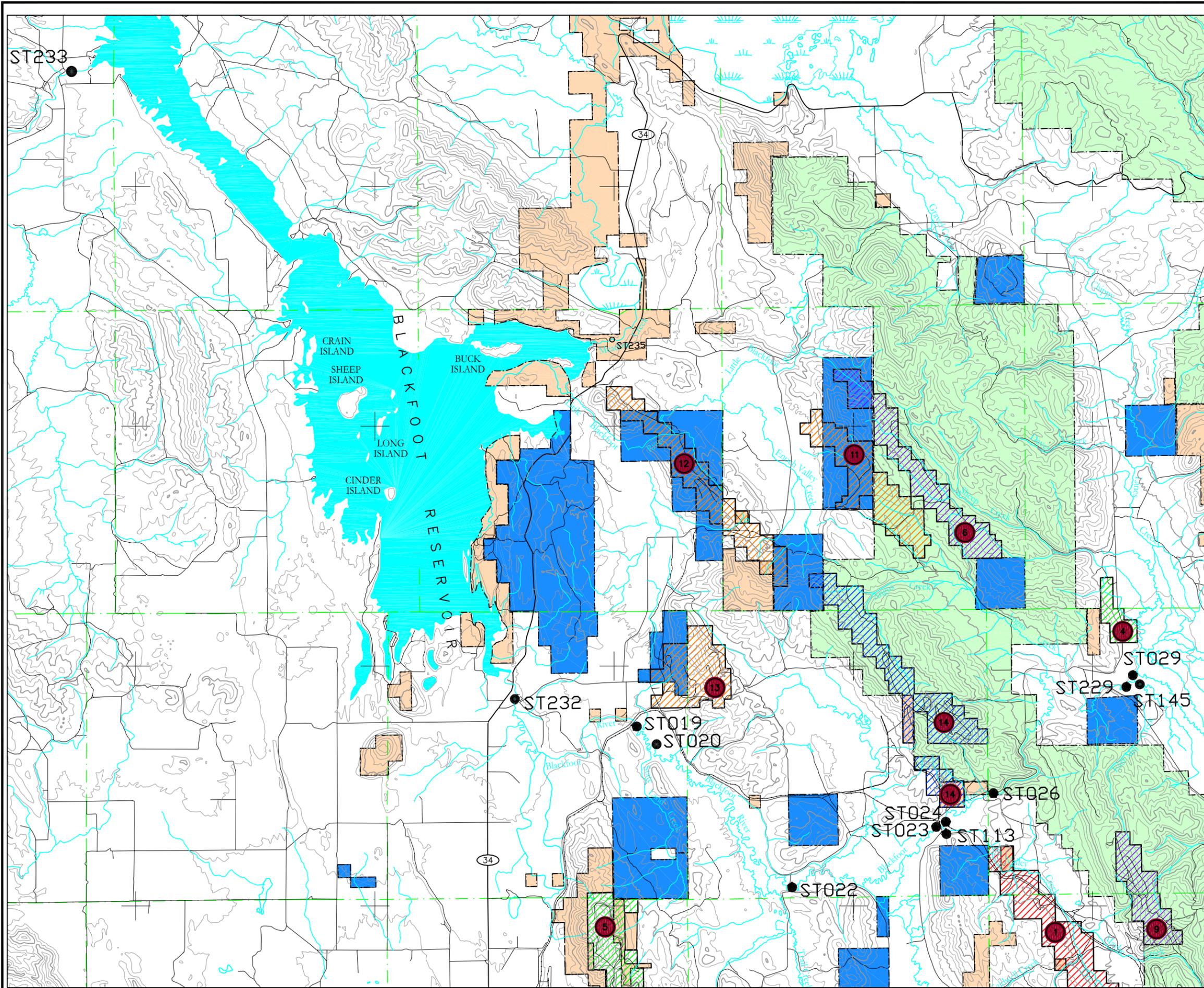
Stream water-column samples were collected from twelve stations in May 1999. Ten of these stations were on the Blackfoot River. One sample each was collected from Dry Valley Creek and Spring Creek. Two locations, ST232 (Blackfoot River upstream of Blackfoot Reservoir) and ST 113 (Dry Valley Creek upstream of Blackfoot River) were sampled monthly June through August. Table 4.2, *Interim Investigation Stream Monitoring Locations*, lists the sampling stations, and the locations of these stations are shown on Drawing 4-2, *Stream Monitoring Locations*.

With the exception of the stations ST233, ST232, and ST029 (Blackfoot River, downstream and upstream of the Blackfoot Reservoir, respectively, and Blackfoot River upstream of Spring Creek), the stations were sampled during the 1998 regional investigation. All of these locations are also being sampled as part of the 1999-2000 regional investigation.

Station ID	Site Description	Notes
ST233	Blackfoot River downstream of Blackfoot Reservoir (immediately upstream of road bridge)	New location
ST232	Blackfoot River, upstream of Blackfoot Reservoir (near the China Hat store, at the Blackfoot Park "sucker trap")	New location; Monthly monitoring site
ST019	Blackfoot River, downstream of Ballard Creek (immediately upstream of Enoch Valley Mine haul road bridge)	
ST020	Blackfoot River, downstream of State Land Creek	
ST022	Blackfoot River, downstream of Wooley Valley Creek (immediately upstream of Trail Creek Road bridge)	
ST023	Blackfoot River, downstream of Dry Valley Creek	
ST024	Blackfoot River, upstream of Dry Valley Creek	
ST026	Blackfoot River, above Wooley Ridge Range Creek (near the upstream mouth of "the Narrows")	
ST229	Blackfoot River, downstream of Spring Creek	
ST029	Blackfoot River, upstream of Spring Creek	New location
ST113	Dry Valley Creek, upstream of Blackfoot River	Monthly monitoring site
ST145	Spring Creek upstream of Blackfoot River	

Water column samples were analyzed for the parameters presented in Table 3.1. The validated results are found in Appendix F. Data validation parameters are also presented in Appendix F.

Following calculation of the validated concentrations, the samples were evaluated to determine at what concentration level a constituent value represented a reportable value. An upper tolerance bound (UTB) on the 95<sup>th</sup> percentile of the distribution of blank results was calculated to distinguish



# LEGEND

- CONTOURS
- CREEKS/RIVERS
- LAKES
- MARSH
- ROADS
- RAILROAD
- STATE LINE
- COUNTY LINE

- NATIONAL FOREST
- BUREAU OF LAND MANAGEMENT
- STATE OF IDAHO
- FORT HALL INDIAN RESERVATION

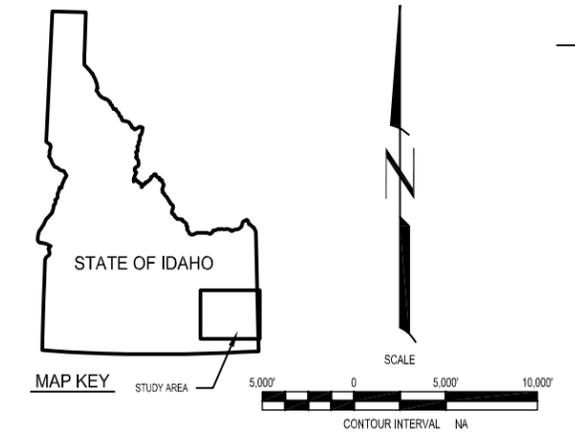
### PHOSPHATE MINES

- ASTARIS PRODUCTION LLC
- FMC CORPORATION AND J.R. SIMPLOT COMPANY
- J.R. SIMPLOT COMPANY
- NU-WEST MINING, INC. OR NU-WEST INDUSTRIES, INC.
- MONSANTO COMPANY
- RHODIA INC.

- ① DRY VALLEY MINE
- ② LANES CREEK MINE
- ③ CONDA MINE
- ④ RASMUSSEN RIDGE MINE
- ⑤ NORTH MAYBE MINE
- ⑥ ENOCH VALLEY MINE
- ⑦ HENRY MINE
- ⑧ BALLARD MINE
- ⑨ WOOLEY VALLEY MINE

### SURFACE WATER INFORMATION

ST233 ● SURFACE WATER MONITORING LOCATION



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0	For 1999 Interim Investigation Data Report	10/20/00	J.Weisman	K.Conrath	J.Weisman

## IDAHO MINING ASSOCIATION SELENIUM COMMITTEE

PROJECT: 1999 INTERIM INVESTIGATION DATA REPORT  
SOUTHEAST IDAHO PHOSPHATE RESOURCE AREA SELENIUM PROJECT

DRAWING TITLE: **STREAM MONITORING LOCATIONS**

**MONTGOMERY WATSON**

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SCALE: AS SHOWN DRAWING No. 4-2

reportable values from those that could not be differentiated from laboratory interference. UTBs were calculated for each constituent concentration and the results are also found in Appendix F.

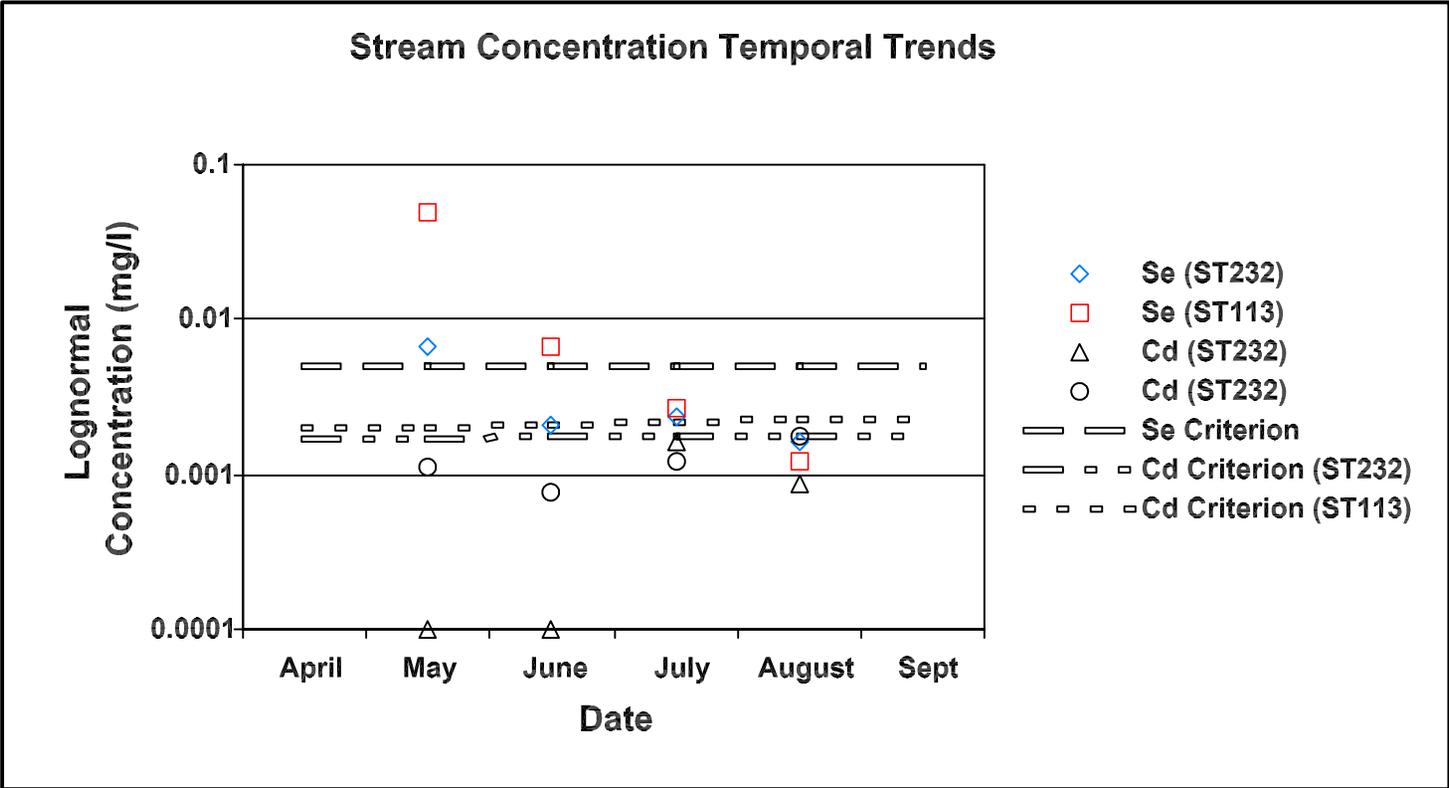
Table 4.3, *Stream Selenium and Cadmium Concentrations*, summarizes the selenium and cadmium results. Bolded values are greater than their corresponding aquatic cold-water criteria, and italicized values are less than their respective UTB.

TABLE 4.3 STREAM SELENIUM AND CADMIUM CONCENTRATIONS					
Site ID	Date	Selenium (mg/L)	Selenium Chronic Cold-Water Criterion <sup>1</sup>	Cadmium (mg/L)	Cadmium Chronic Cold-Water Criterion <sup>2</sup>
May					
ST233	5/26/99	<i>0.00049</i>	0.005	<i>0.0015</i>	0.0019
ST232	5/24/99	<b>0.0067</b>	0.005	<i>-0.00076</i>	0.0017
ST019	5/25/99	<b>0.0082</b>	0.005	<i>-0.00073</i>	0.0015
ST020	5/25/99	<b>0.0072</b>	0.005	<i>-0.00073</i>	0.0015
ST022	5/25/99	<b>0.0098</b>	0.005	<i>0.0</i>	0.0015
ST023	5/24/99	<b>0.0079</b>	0.005	<i>-0.0015</i>	0.0015
ST113	5/24/99	<b>0.049</b>	0.005	<i>0.0011</i>	0.0020
ST024	5/24/99	<b>0.0074</b>	0.005	<i>0.00073</i>	0.0015
ST026	5/25/99	<b>0.0090</b>	0.005	<i>-0.0012</i>	0.0015
ST229	5/25/99	<b>0.019</b>	0.005	<i>-0.00073</i>	0.0015
ST145	5/25/99	<b>0.046</b>	0.005	<i>-0.0015</i>	0.0016
ST029	5/26/99	0.00044	0.005	<i>0.00048</i>	0.0014
June <sup>3</sup>					
ST232	6/23/99	0.0021	0.005	0.00010	0.0018
ST113	6/23/99	<b>0.0068</b>	0.005	0.00078	0.0021
July <sup>3</sup>					
ST232	7/21/99	0.0024	0.005	0.0016	0.0018
ST113	7/21/99	0.0027	0.005	0.0012	0.0022
August <sup>3</sup>					
ST232	8/10/99	0.0015	0.005	0.00088	0.0018
ST113	8/10/99	0.00099	0.005	0.0018	0.0023
<b>Notes:</b> <sup>1</sup> EPA chronic criterion (EPA, 1998). <sup>2</sup> Hardness-specific chronic criterion. Hardness values are presented in Appendix F, Table F-2. <sup>3</sup> Upper Tolerance Bounds (UTBs) were not calculated for June, July and August samples because the sample size was inadequate for statistical tests. The UTB calculations are presented in Appendix F. Bolded values exceed the chronic aquatic cold-water criterion. Italicized values are less than the UTB for the blank samples.					

Drawing 4-3, *Stream Concentration Temporal Trends*, graphically displays selenium and cadmium concentrations from May through August. These data appear to be consistent with what was previously observed in Dry Valley Creek and the Blackfoot River (MW, 1999c). Drawing 4-3 shows that selenium concentrations at both stations exceeded the chronic aquatic cold-water standard of 0.005 mg/L in May. In June, only the Dry Valley Creek sample exceeded the standard, and, by July the selenium concentrations were less than the standard at both stations. Cadmium concentrations at both locations were less than the hardness-specific chronic standard during all sampling events.

The following field parameters were measured:

- pH
- Conductivity
- Temperature
- Dissolved Oxygen
- Turbidity



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REV. No.	REVISIONS	DATE	DESIGN BY	DRAWN BY	REVIEWED AND SIGNED BY
 <b>MONTGOMERY WATSON</b>			PROJECT No.: 1227028.021805 AutoCAD FILE: 1999INTERM.FIG4-3-10-00 SCALE: N.A.      FIGURE No: <b>4-3</b>		

*IDAHO MINING ASSOCIATION  
SELENIUM COMMITTEE*

## **STREAM CONCENTRATION TEMPORAL TRENDS**

- Oxygen-reduction potential
- Flow

Field parameters recorded for each of the monitoring events are presented in Appendix F, Table F-3. Table 4.4, *Stream Field Data*, summarizes the range of field data collected between May and August 1999. These data are consistent with what was observed in 1998 (MW, 1999c).

TABLE 4.4 STREAM FIELD DATA	
Parameter	Range of Reported Values
pH (units)	7.8 – 8.4
Conductivity (µS/cm)	297 – 506
Temperature (°C) <sup>1</sup>	9.8 – 22.1
Dissolved Oxygen (mg/L)	7.7 – 10.9
Turbidity (NTU) <sup>2</sup>	3.93 – 113
Oxygen-Reduction Potential (mV) <sup>3</sup>	97 – 206
Notes: <sup>1</sup> In general, the coolest temperatures were measured in May and increased every month. The 22.1 value was measured at ST232 in July.	
<sup>2</sup> Turbidity readings were typically much higher in May than the other months. Measurements at ST113 were similar each month.	
<sup>3</sup> Oxygen-reduction potential was not measured June through August.	

### 4.3 BIOTA INVESTIGATIONS

This subsection provides data or progress reports for biota investigations done in 1999 or concluded in early 2000.

#### 4.3.1 Bird Egg Investigation

This subsection summarizes findings from the first year of the two-year bird egg study.

The 1999 field season was initiated on May 9th and was terminated on July 9th. Much of the first two weeks were devoted to egg-collection design and area reconnaissance. Bird eggs were collected from areas affected by phosphate mining (i.e., seleniferous habitats) and control areas (i.e., non-seleniferous habitats). Four separate habitat strata were sampled:

- lakes and reservoirs;
- ponds and lacustrine wetlands;
- rivers; and,
- streams.

Two-hundred fifteen (215) eggs were collected, 117 from seleniferous habitat. Eggs were collected from 27 species, with a relatively good distribution among strata and treatments (i.e., seleniferous and non-seleniferous locations). Several species that were on the collecting permit proved to be fairly difficult to locate, or populations were simply low within the study areas (e.g., eared grebe, black tern, house wren).

Table 4.5, *Summary of Bird Eggs Collected from Control Areas in 1999*, identifies how many eggs per stratum, by species, were collected from the control areas. Table 4.6, *Summary of Bird Eggs Collected from Mining Areas in 1999*, identifies how many eggs per stratum, by species, were collected from the phosphate mining area. Drawing 4-4, *1999 Bird Egg Study Nest Locations*, shows where bird eggs, by species, were collected in 1999. The upper Blackfoot River system, Roberts Creek near the Smoky Canyon Mine, and the Blackfoot Reservoir were considered mining-areas.

TABLE 4.5 SUMMARY OF BIRD EGGS COLLECTED FROM CONTROL AREAS IN 1999 <sup>1</sup>					
Common Name	Strata				Total No. of Eggs
	Stream	River	Pond/Wetland	Lake/Reservoir	
American Coot			6	5	11
American Kestrel					0
American Robin	5	1	1	2	9
Barn Swallow					0
Brown-headed Cowbird	5	2	2		9
Canada Goose			2	4	6
Cinnamon Teal			3	1	4
Cliff Swallow	2	5			7
Common Snipe	4				4
European Starling	1				1
Franklin's Gull				5	5
Killdeer	1				1
Long-billed Curlew			1		1
Mallard				2	2
Marsh Wren					0
Mountain Bluebird			1		1
Northern Flicker					0
Red-winged Blackbird		4		5	9
Song Sparrow	5	4	1		10
Tree Swallow			2		2
White-faced Ibis				5	5
Willet					0
Yellow-headed Blackbird		1	5	1	7
Total No. Eggs per strata	23	17	25	33	98

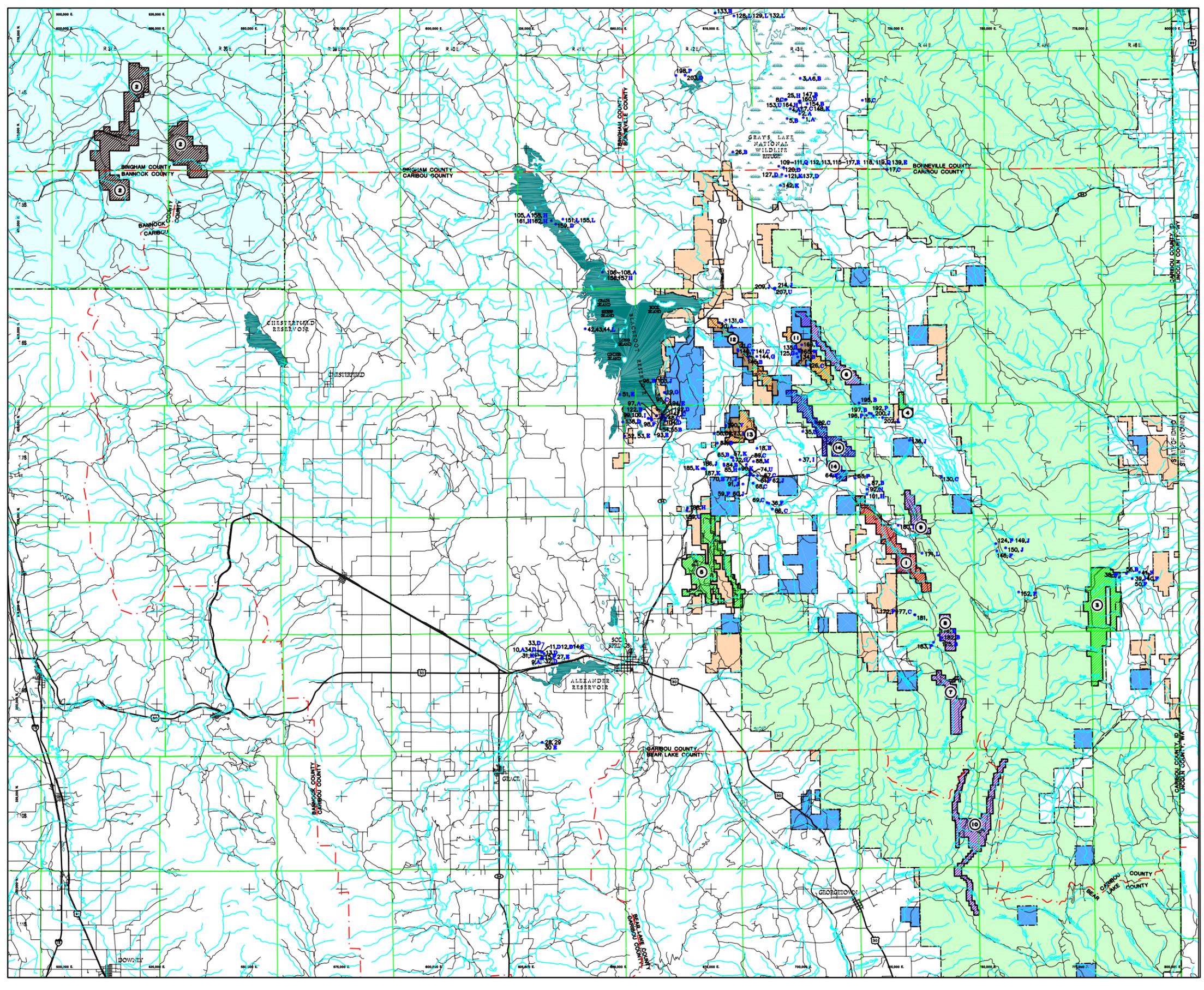
Notes: <sup>1</sup>Blank cells indicates that an egg for that species was not collected for that stratum.

TABLE 4.6 SUMMARY OF AVIAN EGGS COLLECTED FROM MINING AREAS IN 1999 <sup>1</sup>					
Common Name	Strata				Total No. of Eggs
	Stream	River	Pond/Wetland	Lake/Reservoir	
American Coot				5	5
American Kestrel			1		1
American Robin	2	6	5	2	15
Barn Swallow	1			2	11
Brown-headed Cowbird	2	4	2	1	9
Canada Goose			1	5	6
Cinnamon Teal		2	1		3
Cliff Swallow	3	3		5	11
Common Snipe	3				3
European Starling	1	1	1		3
Franklin's Gull					0
Killdeer		1			1
Long-billed Curlew					0
Mallard		2	2	5	9
Marsh Wren		2	2		4
Mountain Bluebird			5	1	6
Northern Flicker	1		1		2
Red-winged Blackbird	5	4	4	5	18
Song Sparrow	2	5	2	1	10
Tree Swallow			2		2
White-faced Ibis					0
Willet			1		1
Yellow-headed Blackbird				5	5
Total No. eggs per strata	20	30	30	37	117

Notes: <sup>1</sup>Blank cells indicates that an egg for that species was not collected for that stratum.

Drawing 4-4, 1999 Bird Egg Study Nest Locations

AutoCAD FILE: 028-EGG-01.dwg PROJECT NUMBER: 227028-0218/05



# LEGEND

- CONTOURS
- CREEKS/RIVERS
- LAKES
- MARSH
- ROADS
- RAILROAD
- STATE LINE
- COUNTY LINE
- NATIONAL FOREST
- BUREAU OF LAND MANAGEMENT
- STATE OF IDAHO
- FORT HALL INDIAN RESERVATION
- PHOSPHATE MINES**
- ASTARIS PRODUCTION LLC
- FMC CORPORATION AND J.R. SIMPLOT COMPANY
- J.R. SIMPLOT COMPANY
- NU-WEST MINING, INC. OR NU-WEST INDUSTRIES, INC.
- MONSANTO COMPANY
- RHODIA INC.

- ① DRY VALLEY MINE
- ② GAY MINE
- ③ SMOKY CANYON MINE
- ④ LANES CREEK MINE
- ⑤ CONDA MINE
- ⑥ RASMUSSEN RIDGE MINE
- ⑦ MOUNTAIN FUEL MINE
- ⑧ CHAMP MINE
- ⑨ NORTH MAYBE MINE
- ⑩ GEORGETOWN CANYON MINE
- ⑪ ENOCH VALLEY MINE
- ⑫ HENRY MINE
- ⑬ BALLARD MINE
- ⑭ WOOLEY VALLEY MINE

### BIRD EGG INFORMATION

- A. CANADA GOOSE
- B. RED-WINGED BLACKBIRD
- C. AMERICAN ROBIN
- D. AMERICAN COOT
- E. YELLOW-HEADED BLACKBIRD
- F. SONG SPARROW
- G. MOUNTAIN BLUEBIRD
- H. MALLARD
- I. BARN SWALLOW
- J. BROWN-HEADED COWBIRD
- K. MARSH WREN
- 152 SAMPLE ID NUMBER
- L. CLIFF SWALLOW
- M. KILLDEER
- N. WILLET
- O. TREE SWALLOW
- P. COMMON SNIFE
- Q. WHITE-FACED IBIS
- R. FRANKLIN'S GULL
- S. LONG-BILLED CURLEW
- T. AMERICAN KESTREL
- U. CINNAMON TEAL
- V. EUROPEAN STARLING
- W. NORTHERN FLICKER



REV. NO.	REVISIONS	DATE	DESIGN BY	DRAWN BY	REVIEWED AND SIGNED BY
0	For 1999 Interim Investigation Data Report	10/20/00	J.Weinman	T.Clark	J.Weinman

## IDAHO MINING ASSOCIATION SELENIUM COMMITTEE

PROJECT: 1999 INTERIM INVESTIGATION DATA REPORT  
SOUTHEAST IDAHO PHOSPHATE RESOURCE AREA SELENIUM PROJECT  
DRAWING TITLE:  
**1999 BIRD EGG STUDY NEST LOCATIONS**

MONTGOMERY WATSON

Sheet 1 of 1 Sheets  
SCALE: As Shown DRAWING No. 4-4

Gray's Lake National Wildlife Refuge and vicinity, Alexander Reservoir, and upper Diamond Creek were considered control areas. Samples were analyzed for the parameters presented in Table 3.2.

Eggs from 18 different species were sampled in the control area, while 20 species were sampled in the mine area. Selenium levels among species were compared using lognormal fit plots and a preliminary three-way analysis of variance. A review of the preliminary data indicates that seven of 20 species from the mining area species had selenium concentrations in some eggs that exceeded 10 mg/kg (dry), a recommended preliminary toxicity benchmark. Fifteen (15) of 117 eggs (12.8 percent) collected from the mining areas exceeded 10 mg/kg (dry). The species with egg selenium concentrations greater than 10 mg/kg (dry) were:

- yellow-headed blackbird;
- common snipe;
- European starling;
- mountain bluebird;
- red-winged blackbird;
- American kestrel; and,
- song sparrow.

However, it is important to note that these eggs are from only one year of egg collection. Sample sizes are relatively small, and, although some egg selenium levels were elevated in the mining area, most were within (or close to) the background range reported for many species in other regions by other researchers.

Field teams noted several species that were not included in collecting permit for 1999 that were fairly abundant, and whose nests were easily located. These included yellow warbler, western grebe, Brewer's blackbird, sandhill crane, double-crested cormorant, California gull, and ring-billed gull. These species have been added to the 2000 field season collecting permit from the USFWS and IDFG.

To determine if the observed elevated selenium concentrations are potentially detrimental to ecological health, a reproductive success study will be initiated during the 2000 field study. This study will look at key reproductive parameters including clutch size, hatching success, fledging success, and post-fledging survival to see if a reduction in reproductive success can be observed. Information will be collected on five or six species. Potential target species include:

- American Coot;
- American Robin;
- barn swallow;
- red-winged blackbird; and,
- yellow-headed blackbird.

Field teams will monitor approximately 20 nests of each species from both the control and mine areas. These species were selected because they are common in the study area, their nests are easily found and monitored (Rocklage, et al., 2000), they rarely desert nests due to human interference (Ortega et al., 1997), and they are rarely parasitized by brown-headed cowbirds (Ehrlich et al., 1998). The reproductive success task protocols are presented in Appendix G.

#### 4.3.2 Elk Investigation

The IMA Selenium Committee and IDFG cooperated to collect elk tissue samples from elk hunters who harvested from game management units 76 and 66A. At the time the elk study was proposed,

IDFG estimated that the total sample size might include up to 200 skeletal muscle and 30 liver samples (J. Hanson, personal communication, 1999). The estimate was based on the expected 1999 harvest rate and historic check station data.

The total number of animals sampled was very close to IDFG's before-the-fact estimate. Approximately 229 different elk were sampled. Skeletal muscle and liver samples were collected from 94 animals, while liver-only samples were collected from 90 animals. An additional 45 muscle-only samples were also collected. The total number of elk tissue samples collected by IDFG was approximately 323.

The Selenium Committee committed to analyze 250 individual samples. It was decided to analyze all elk with both liver and muscle tissue to determine if a correlation existed between liver and muscle concentrations. Two samples with both tissues were not analyzed because they had the same license number (101-9-00113). It was not possible to determine if sample might be mislabeled, so to eliminate any potential bias neither sample was analyzed. Sample 202-9-502746 was received at the laboratory with two muscle samples. Both muscles samples were analyzed and the results averaged during the data evaluation process. Consequently, the total number of animals with both liver and muscle tissues that were analyzed was 91. A random sample of the liver-only elk was selected to fill out the number of analysis to 250. Thus, 160 liver and 91 muscle samples were analyzed.

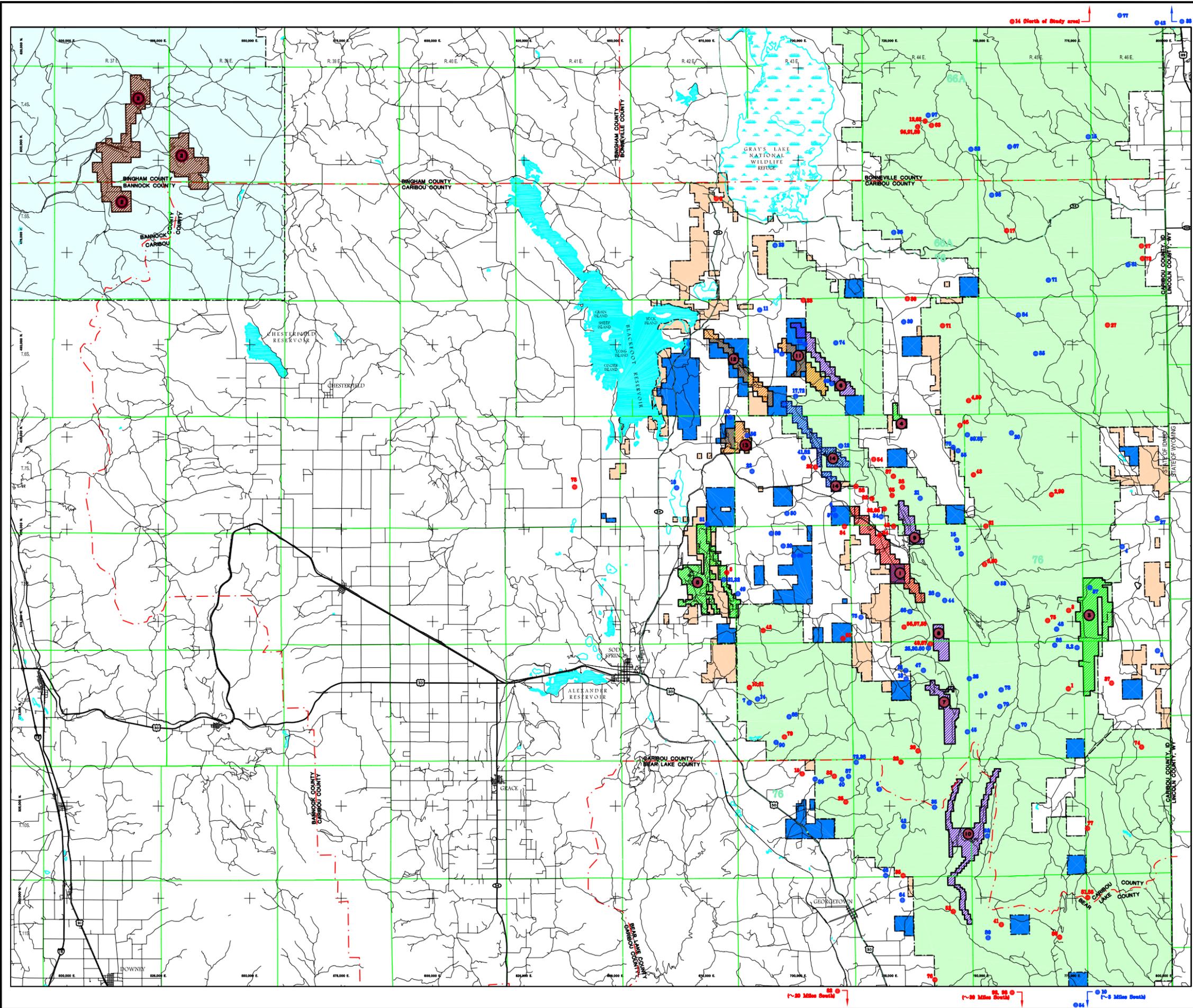
The tissue samples were analyzed for the parameters identified in Table 3.3. Validated results are found in Appendix H. Data validation parameters are also included in Appendix H. Following calculation of the validated concentrations, the samples were evaluated to determine at what concentration level a constituent value represented a reportable value. An UTB on the 95<sup>th</sup> percentile of the distribution of blank results was calculated to distinguish reportable values from those that could not be differentiated from laboratory interference. UTBs were calculated for each constituent concentration and the results are also found in Appendix H.

Table 4.7, *Elk Liver and Muscle Selenium and Cadmium Concentrations*, summarizes selenium and cadmium concentration data. Italicized values are less than their corresponding UTB value. Drawing 4-5, *1999 Elk Study Kill Locations*, shows the approximate kill location of each animal sampled.

In the late 1970's and early 1980's the IDFG conducted a baseline study in southeast Idaho of phosphate mining impacts on elk (Kuck, 1984). The baseline study indicated that movement patterns were nomadic and that home areas could best be described as "elliptical polygons." Kuck (1984) reported there was substantial variation between individuals and years. The mean year-around home range for elk was reported as 26 square miles, with a mean migration distance between summer and winter range of 4.1 miles.

The Selenium Committee used the IDFG information to classify elk before-the-fact (i.e., before any laboratory results were received) as either a control or mine-area elk. Any elk killed at a distance of 10 or more miles (3.4 mean home-range radii) from a phosphate mine were classified as before-the-fact controls. Those killed at closer distances to mines were classified before-the-fact as mine-area elk. The study null hypothesis was that there would be no difference in the mean selenium concentrations in liver and skeletal muscle tissue in elk harvested from the mine-area when compared to control-area tissue.

Twenty-seven liver and 14 muscle samples were classified as before-the fact control, as indicated in Table 4.7.



# LEGEND

- CONTOURS
- CREEKS/RIVERS
- LAKES
- MARSH
- ROADS
- RAILROAD
- STATE LINE
- COUNTY LINE

- NATIONAL FOREST
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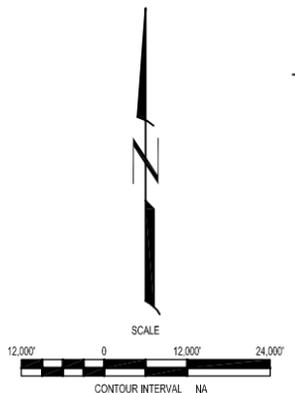
### PHOSPHATE MINES

- ASTARIS PRODUCTION LLC
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- MONSANTO COMPANY
- RHODIA INC.

- 1 DRY VALLEY MINE
- 2 GAY MINE
- 3 SMOKY CANYON MINE
- 4 LANES CREEK MINE
- 5 CONDA MINE
- 6 RASMUSSEN RIDGE MINE
- 7 MOUNTAIN FUEL MINE
- 8 CHAMP MINE
- 9 NORTH MAYBE MINE
- 10 GEORGETOWN CANYON MINE
- 11 ENOCH VALLEY MINE
- 12 HENRY MINE
- 13 BALLARD MINE
- 14 WOOLEY VALLEY MINE

### ELK INFORMATION

- ELK LIVER TISSUE SAMPLE
- ELK LIVER AND MUSCLE TISSUE SAMPLE
- GAME MANAGEMENT UNIT BOUNDARY
- 76 GAME MANAGEMENT UNIT BOUNDARY



0	For 1999 Interim Investigation Data Report	10/20/00	J.Welman	T.Clark	J.Welman
REV. No.	REVISIONS	DATE	DESIGN BY	DRAWN BY	REVIEWED AND SIGNED BY

## IDAHO MINING ASSOCIATION SELENIUM COMMITTEE

PROJECT: 1999 INTERIM INVESTIGATION DATA REPORT  
SOUTHEAST IDAHO PHOSPHATE RESOURCE AREA SELENIUM PROJECT  
DRAWING TITLE:  
**1999 ELK STUDY KILL LOCATIONS**



Sheet 1 Of 1 Sheets  
SCALE: As Shown  
DRAWING No. 4-5

PROJECT NUMBER: 227028.02504  
AUTOCAD FILE: 028-ELK.dwg

**TABLE 4.7  
ELK LIVER AND MUSCLE SELENIUM AND CADMIUM CONCENTRATIONS**

IDFG License Number <sup>1</sup>	Sample Type		Map ID	General Kill Location	Before-the-Fact Treatment <sup>2</sup>	Selenium				Cadmium				After-the-Fact Treatment <sup>3</sup>
						Liver		Skeletal Muscle		Liver		Skeletal Muscle		
	Liver	Muscle				Wet-Weight (mg/kg)	Dry-Weight (mg/kg)							
101-9-001192	X	X	2	Sage Creek	M	0.53	1.7	0.12	0.44	0.39	1.3	0.084	0.31	C
101-9-003717	X	X	3	Buck Mountain	M	13	38	0.36	1.2	0.26	0.79	0.037	0.12	E
101-9-003744	X	X	4	Draney Creek	M	0.29	1.0	0.10	0.39	0.14	0.51	0.060	0.24	C
101-9-003781	X	X	5	Rattlesnake Canyon	M	0.34	1.2	0.11	0.36	0.13	0.47	0.049	0.16	C
101-9-003836	X	X	6	Dry Valley	M	0.98	3.2	0.22	0.78	0.66	2.2	0.075	0.27	C
101-9-005127	X	X	7	South Sulpher Canyon	M	0.50	1.5	0.21	0.69	0.65	2.0	0.099	0.33	C
101-9-007510	X	X	8	Sage Creek	M	1.3	4.3	0.12	0.43	0.31	1.0	0.155	0.58	C
101-9-008142	X	X	9	Dry Valley	M	1.3	4.7	0.17	0.59	0.43	1.6	0.074	0.26	C
101-9-009603	X	X	10	Pruess Creek	C	0.59	2.2	0.14	0.48	0.29	1.1	0.056	0.20	C
101-9-010800	X	X	11	Long Valley	M	0.26	1.0	0.10	0.41	0.29	1.1	0.071	0.29	C
101-9-011498	X	X	89	Trout Creek	M	0.49	1.9	0.063	0.24	0.32	1.2	0.050	0.19	C
101-9-011744	X	X	12	Rassmussen Valley	M	1.9	6.7	0.15	0.64	0.37	1.3	0.029	0.12	C
101-9-013229	X	X	13	Slug Creek	M	5.3	19	0.37	1.2	0.50	1.8	0.122	0.38	E
101-9-015568	X	X	14	South Sulpher Canyon	M	0.39	1.4	0.12	0.45	0.65	2.3	0.051	0.19	C
101-9-016571	X	X	15	Jacknife Creek	C	0.64	2.1	0.14	0.54	0.49	1.6	0.042	0.17	C
101-9-018227	X	X	16	Kendall Canyon	M	2.0	7.6	0.33	1.1	0.36	1.3	0.068	0.22	E
101-9-020202	X	X	17	Wooley Range	M	0.41	1.5	0.13	0.48	0.30	1.1	0.036	0.14	C
101-9-020457	X	X	18	Woodall Spring	M	1.2	4.2	0.15	0.64	0.42	1.5	0.108	0.45	C
101-9-022067	X	X	19	Hornet Canyon	M	0.54	1.9	0.13	0.42	0.24	0.83	0.067	0.22	C
101-9-024783	X	X	20	Diamond Flat	M	1.6	5.2	0.43	1.9	0.51	1.7	0.052	0.23	E
101-9-024889	X	X	21	Hornet Canyon	M	0.28	0.87	0.11	0.40	0.25	0.79	0.048	0.18	C
101-9-025926	X	X	22	Fox Hills	M	1.1	3.5	0.29	1.1	0.37	1.2	0.052	0.20	C
101-9-026635	X	X	23	Little Grey Ridge	M	0.22	0.73	0.10	0.32	0.59	2.0	0.146	0.47	C
101-9-028332	X	X	83	Morgan Meadows	C	0.71	2.3	0.15	0.57	0.35	1.1	0.108	0.40	C
101-9-028637	X	X	24	Upper Enoch Valley	M	1.2	4.2	0.27	0.97	1.2	4.1	0.127	0.45	E
101-9-033823	X	X	96	Tincup Mountain	C	0.68	2.3	0.15	0.51	0.34	1.1	0.165	0.57	C
101-9-034278	X	X	25	Dry Canyon	M	3.2	12	0.24	0.87	0.33	1.2	0.137	0.51	E
101-9-041577	X	X	26	Upper Dry Valley	M	2.7	9.1	0.21	0.74	0.37	1.2	0.184	0.66	E
101-9-045262	X	X	27	Tygee Creek	M	4.6	15	0.48	1.8	0.49	1.6	0.278	1.03	E
101-9-048459	X	X	28	Dry Valley	M	5.3	18	0.79	2.9	0.34	1.1	0.137	0.51	E
101-9-054687	X	X	29	Trout Creek	M	0.34	1.1	0.12	0.43	1.6	5.2	0.074	0.27	C
101-9-057366	X	X	30	Dry Canyon	M	3.4	9.7	0.18	0.60	0.60	1.7	0.137	0.46	E
101-9-064273	X	X	31	Shield Canyon	M	2.6	9.2	0.52	2.0	0.43	1.5	0.066	0.25	E
101-9-065581	X	X	32	N. Trail Canyon Road	M	2.2	8.6	0.35	1.3	0.35	1.4	0.108	0.40	E
101-9-066053	X	X	88	Sage Meadows	M	2.4	8.8	0.34	1.1	0.39	1.4	0.108	0.34	E
101-9-067130	X	X	69	Rassmussen Ridge	M	2.0	6.4	0.17	0.72	0.43	1.4	0.146	0.61	E
101-9-067901	X	X	33	McCoy Creek	C	0.47	1.6	0.093	0.33	0.33	1.1	0.057	0.20	C
101-9-069187	X	X	34	Dry Valley	M	2.5	13	0.19	0.76	0.21	1.1	0.022	0.09	E
101-9-069462	X	X	35	Kendall Canyon	M	0.43	1.3	0.15	0.49	1.1	3.3	0.174	0.56	C
101-9-072990	X	X	36	Bridge Creek	C	1.5	5.3	0.11	0.40	0.37	1.3	0.015	0.06	C
101-9-075567	X	X	37	Smoky Canyon	M	0.62	2.2	0.20	0.75	0.29	1.0	0.033	0.12	C

**TABLE 4.7  
ELK LIVER AND MUSCLE SELENIUM AND CADMIUM CONCENTRATIONS**

IDFG License Number <sup>1</sup>	Sample Type		Map ID	General Kill Location	Before-the-Fact Treatment <sup>2</sup>	Selenium				Cadmium				After-the-Fact Treatment <sup>3</sup>
						Liver		Skeletal Muscle		Liver		Skeletal Muscle		
	Liver	Muscle				Wet-Weight (mg/kg)	Dry-Weight (mg/kg)							
101-9-081257	X	X	38	Jones Canyon	M	0.35	1.0	0.085	0.28	1.7	4.7	0.071	0.23	C
101-9-081312	X	X	39	Chippy Creek	M	0.51	1.8	0.13	0.41	0.32	1.1	0.040	0.13	C
101-9-082092	X	X	40	Jones Canyon	M	0.48	1.8	0.14	0.48	0.73	2.7	0.0095	0.03	C
101-9-082893	X	X	41	Wooley Valley	M	0.79	2.8	0.12	0.43	0.21	0.72	0.0050	0.02	C
101-9-083966	X	X	42	Burns Creek	C	0.33	0.93	0.085	0.24	0.23	0.65	0.0018	0.00	C
102-9-003525	X	X	43	Summit View Campground	M	0.31	1.1	0.12	0.43	0.19	0.67	0.127	0.47	C
102-9-007374	X	X	44	Dry Valley Ridge	M	7.9	28	0.41	1.6	0.33	1.2	0.095	0.37	E
102-9-008730	X	X	45	Upper Dry Valley	M	2.3	8.0	0.66	2.4	0.76	2.6	0.137	0.51	E
102-9-009130	X	X	46	Left Hand Fork Georgetown Creek	M	1.1	3.7	0.16	0.51	0.46	1.6	0.174	0.54	C
102-9-009184	X	X	47	Schmid Ridge	M	1.7	5.7	0.18	0.63	0.15	0.52	0.068	0.24	C
102-9-010135	X	X	97	Upper Tincup Creek	C	0.36	1.2	0.12	0.47	0.29	1.0	0.0018	0.01	C
102-9-010277	X	X	55	Timothy Creek	M	0.50	1.7	0.060	0.23	0.23	0.80	-0.0077	-0.03	C
102-9-010303	X	X	48	Pole Canyon	M	5.8	21	0.34	1.3	0.21	0.77	-0.016	-0.06	E
102-9-011973	X	X	49	Pedro Creek	M	0.33	1.1	0.076	0.28	0.43	1.5	0.159	0.59	C
102-9-012019	X	X	50	Lower Valley	M	0.63	2.1	0.14	0.52	0.29	1.0	0.0086	0.03	C
104-9-001284	X	X	87	Jones Creek	M	0.57	2.1	0.12	0.47	0.25	0.90	0.013	0.05	C
104-9-004294	X	X	51	Woodall Mountain	M	1.6	5.6	0.92	3.4	0.45	1.6	0.118	0.44	E
104-9-005505	X	X	52	Snowdrift Mountain	M	5.7	19	0.29	1.1	0.20	0.66	-0.021	-0.08	E
104-9-006297	X	X	53	Campbell Canyon	M	5.6	19	0.42	1.6	0.20	0.68	0.118	0.44	E
104-9-006924	X	X	54	Preuss Creek	C	0.32	1.1	0.076	0.25	0.16	0.57	0.099	0.33	C
104-9-009499	X	X	86	Jones Canyon	M	1.2	4.0	0.14	0.60	0.30	1.0	0.021	0.09	C
104-9-014257	X	X	56	Fox Hills	M	0.99	3.8	0.54	1.7	0.48	1.8	0.127	0.41	E
105-9-000606	X	X	57	Dry Valley	M	1.1	3.8	0.21	0.77	0.62	2.2	0.019	0.07	C
105-9-004447	X	X	58	Long Valley	M	2.7	9.1	0.19	0.70	0.48	1.6	0.174	0.62	E
105-9-006076	X	X	59	Timothy Creek	M	0.27	1.0	0.072	0.29	0.22	0.80	-0.012	-0.05	C
105-9-006125	X	X	60	Dry Valley	M	6.3	19	0.26	0.94	0.42	1.3	0.00034	0.00	E
106-9-000834	X	X	61	Deer Creek	C	0.88	2.7	0.13	0.48	0.61	1.9	0.118	0.45	C
106-9-001250	X	X	62	Upper Rasmussen Valley	M	1.9	6.1	0.33	1.3	0.76	2.4	0.010	0.04	C
106-9-006628	X	X	63	Schmid Ridge	M	0.47	1.6	0.13	0.48	1.5	5.1	0.0053	0.02	C
106-9-009470	X	X	64	East Hill	M	0.76	2.4	0.18	0.72	0.46	1.5	0.137	0.55	C
106-9-010309	X	X	65	Bacon Creek	M	0.37	1.3	0.18	0.63	0.21	0.74	0.012	0.04	C
107-9-001328	X	X	66	Campbell Canyon	M	0.38	1.1	0.16	0.63	0.65	1.9	0.146	0.56	C
107-9-002807	X	X	67	Jacknife Creek	C	0.30	1.1	0.12	0.49	0.59	2.1	0.035	0.15	C
107-9-007918	X	X	68	Diamond Gulch	M	0.74	2.4	0.13	0.48	0.90	2.9	0.048	0.18	C
107-9-008536	X	X	82	Wooley Valley	M	0.37	1.2	0.18	0.67	0.30	1.0	0.075	0.28	C
108-9-001128	X	X	90	Diamond Gulch	M	0.57	1.6	0.16	0.54	0.65	1.9	0.108	0.36	C
108-9-001232	X	X	70	North Deer Creek	M	0.61	2.2	0.20	0.74	0.53	1.9	0.024	0.09	C
108-9-003843	X	X	71	South Fork Creek	C	0.36	1.2	0.14	0.44	0.24	0.84	0.080	0.26	C

**TABLE 4.7  
ELK LIVER AND MUSCLE SELENIUM AND CADMIUM CONCENTRATIONS**

IDFG License Number <sup>1</sup>	Sample Type		Map ID	General Kill Location	Before-the-Fact Treatment <sup>2</sup>	Selenium				Cadmium				After-the-Fact Treatment <sup>3</sup>
						Liver		Skeletal Muscle		Liver		Skeletal Muscle		
	Liver	Muscle				Wet-Weight (mg/kg)	Dry-Weight (mg/kg)							
108-9-004804	X	X	72	Wooley Range	M	2.6	8.2	0.15	0.55	0.52	1.6	0.084	0.30	E
108-9-005208	X	X	73	Jones Canyon	M	0.31	1.2	0.17	0.62	0.30	1.1	0.099	0.37	C
108-9-005350	X	X	74	Olsen Creek	M	1.2	4.0	0.14	0.54	0.24	0.84	0.118	0.46	C
108-9-005351	X	X	75	North of Wolf Mountain	M	0.63	1.9	0.12	0.49	0.91	2.8	0.025	0.10	C
112-9-000623	X	X	81	Dry Canyon	M	3.2	11	0.34	1.5	0.57	2.0	0.059	0.26	E
125-9-001720	X	X	78	Freeman Ridge	M	0.47	1.5	0.11	0.38	0.32	1.0	0.065	0.23	C
125-9-002440	X	X	79	Freeman Ridge	M	0.70	2.5	0.19	0.63	0.43	1.5	0.108	0.36	C
202-9-003457	X	X	84	North Fork Stump Creek	C	0.40	1.3	0.071	0.28	0.39	1.3	0.137	0.55	C
202-9-005862	X	X	76	Timothy Creek	M	0.51	1.5	0.091	0.35	0.32	1.0	0.097	0.37	C
202-9-007411	X	X	77	Black Mountain	M	0.21	0.68	0.077	0.29	0.36	1.2	0.074	0.27	C
202-9-502746	X	X	85	Stump Peak	C	0.28	0.93	0.14	0.40	0.68	2.3	0.079	0.23	C
202-9-502746		X	85	Stump Peak	C			0.12	0.32			0.091	0.25	C
803-9-00600	X	X	80	Crow Creek	M	0.49	1.6	0.12	0.45	0.32	1.0	0.077	0.29	C
101-9-000540	X		22	Harrington Peak	M	0.51	1.8			0.29	0.99			
101-9-001509	X		59	Upper Bacon Creek	M	0.34	0.99			0.32	0.93			
101-9-006611	X		77	Warm Creek	M	0.93	3.2			0.69	2.4			
101-9-006617	X		37	Sage Valley	M	0.38	1.2			0.32	1.0			
102-9-009231	X		71	Corraisen Creek	M	0.39	1.3			0.22	0.75			
101-9-009926	X		63	North Fork Tincup Creek	C	0.55	1.7			0.40	1.2			
101-9-012050	X		83	Horseshoe Spring	M	0.59	2.3			0.26	0.99			
101-9-018310	X		85	South Side of Red Mountain	M	0.71	2.4			0.32	1.1			
101-9-018408	X		74	Rock Creek	M	0.53	1.8			0.33	1.2			
101-9-019555	X		50	Wolf Mountain	M	0.69	2.4			0.41	1.4			
101-9-020486	X		78	Chain Hat	M	0.74	2.6			0.23	0.79			
101-9-020534	X		76	Maple Canyon	M	0.47	1.6			0.33	1.2			
101-9-021815	X		27	Smith Creek	M	0.40	1.3			0.24	0.81			
101-9-023207	X		32	Dry Valley	M	3.8	13			0.43	1.4			
101-9-025256	X		28	Henry Peak	M	0.38	1.1			0.51	1.5			
101-9-026427	X		93	Boundary Ridge	C	0.35	1.2			0.22	0.75			
101-9-032948	X		14	Black Mountain	C	0.37	1.3			0.31	1.1			
101-9-035622	X		20	Upper Slug Creek	M	4.7	18			0.38	1.5			
101-9-040872	X		33	Dry Ridge	M	1.4	4.5			0.57	1.9			
101-9-041629	X		98	Boundary Ridge	C	0.26	0.92			0.16	0.57			
101-9-050246	X		46	Bacon Creek	M	0.29	1.1			0.14	0.49			
101-9-052544	X		42	Trail Creek Warming Hut	M	0.28	1.1			0.37	1.4			
101-9-055718	X		73	Deer Creek	C	0.37	1.2			0.25	0.81			
101-9-057289	X		1	Right Fork Deer Creek	M	0.59	1.9			0.37	1.2			
101-9-059906	X		81	Southwest Side of Red Mountain	M	0.90	3.0			0.22	0.75			

**TABLE 4.7  
ELK LIVER AND MUSCLE SELENIUM AND CADMIUM CONCENTRATIONS**

IDFG License Number <sup>1</sup>	Sample Type		Map ID	General Kill Location	Before-the-Fact Treatment <sup>2</sup>	Selenium				Cadmium				After-the-Fact Treatment <sup>3</sup>
						Liver		Skeletal Muscle		Liver		Skeletal Muscle		
	Liver	Muscle				Wet-Weight (mg/kg)	Dry-Weight (mg/kg)							
101-9-060482	X		80	Southwest Side of Red Mountain	M	0.43	1.5			0.25	0.87			
101-9-061415	X		48	Dry Valley	M	5.2	19			0.32	1.2			
101-9-062127	X		87	Dry Ridge	M	0.47	1.6			0.24	0.85			
101-9-062802	X		5	Shield Canyon	M	1.9	6.6			0.31	1.1			
101-9-063339	X		89	Tincup Creek	C	0.26	0.99			0.42	1.6			
101-9-069460	X		25	Big Canyon	M	9.1	33			0.70	2.5			
101-9-072423	X		6	Coyote Creek	M	0.38	1.3			0.15	0.52			
101-9-073654	X		3	Smoky Canyon	M	0.41	1.4			0.16	0.55			
101-9-074150	X		41	Meade Peak	M	0.39	1.4			0.17	0.58			
101-9-074844	X		95	Eagle Creek	C	0.34	0.86			0.43	1.1			
101-9-075445	X		75	Smoky Canyon	M	0.34	1.2			0.24	0.87			
101-9-082849	X		35	Church Hollow	M	0.78	2.4			0.17	0.52			
101-9-084603	X		58	Wolf Mountain	M	4.4	15			0.85	2.8			
102-9-000978	X		52	Dunn Canyon	M	0.17	0.66			0.15	0.58			
102-9-003510	X		62	North Fork Tincup Creek	C	0.23	0.82			0.28	1.0			
102-9-005611	X		29	Wooley Range	M	2.9	8.1			0.66	1.8			
102-9-008618	X		64	Schmid Ridge	M	3.6	12			0.32	1.0			
102-9-009651	X		38	Dry Valley	M	3.7	17			0.24	1.1			
102-9-012285	X		67	Schmid Ridge	M	1.5	5.6			0.46	1.8			
104-9-007653	X		82	Bloomington Canyon	C	0.55	1.9			0.20	0.68			
104-9-008160	X		47	Deer Creek	C	0.37	3.7			0.32	3.2			
104-9-009086	X		30	Middle Dairy	M	0.31	1.2			0.15	0.60			
104-9-011485	X		54	Blackfoot River Wildlife Area	M	2.7	19			0.42	3.0			
106-9-002669	X		40	Schmid Ridge	M	0.60	2.1			0.36	1.3			
106-9-007118	X		43	Timothy	M	0.31	1.0			0.15	0.50			
106-9-009604	X		60	Coyote Creek	M	0.55	2.0			0.22	0.80			
106-9-009855	X		15	Fossil Canyon	M	1.3	3.8			0.41	1.2			
107-7-61023	X		79	Diamond Creek	M	0.20	0.84			0.21	0.86			
107-9-002888	X		66	Dry Ridge	M	3.1	11			0.32	1.1			
107-9-007770	X		4	Upper Bacon Creek	M	0.26	0.86			0.20	0.66			
108-9-001165	X		34	Schmid Ridge	M	4.1	15			0.27	0.96			
108-9-001269	X		65	Dry Ridge	M	1.7	5.9			0.55	2.0			
108-9-004060	X		91	Tincup Creek	C	3.5	12			0.50	1.8			
108-9-004918	X		88	The Narrows	M	0.27	0.96			0.37	1.3			
116-9-9108	X		57	Wolf Mountain	M	2.9	9.4			0.50	1.6			
116-9-9109	X		56	Wolf Mountain	M	2.8	9.4			0.20	0.66			
125-9-000899	X		10	Upper Sulphur Canyon	M	0.44	1.6			0.22	0.80			
125-9-001223	X		17	Caribou Guard Station	C	7.4	25			0.34	1.2			
202-9-003468	X		2	Diamond Flat	M	0.31	1.1			0.20	0.67			

**TABLE 4.7  
ELK LIVER AND MUSCLE SELENIUM AND CADMIUM CONCENTRATIONS**

IDFG License Number <sup>1</sup>	Sample Type		Map ID	General Kill Location	Before-the-Fact Treatment <sup>2</sup>	Selenium				Cadmium				After-the-Fact Treatment <sup>3</sup>
						Liver		Skeletal Muscle		Liver		Skeletal Muscle		
	Liver	Muscle				Wet-Weight (mg/kg)	Dry-Weight (mg/kg)							
202-9-003786	X		99	Diamond Flat	M	0.26	1.0			0.21	0.86			
202-9-508864	X		21	Diamond Creek	M	0.48	1.7			0.11	0.39			
218-9-000273	X		61	Upper Sulphur Canyon	M	1.2	4.0			0.23	0.80			
352-9-000257	X		9	Little Grey Ridge	M	0.25	0.80			0.23	0.75			
803-9-015407	X		12	North Fork Tincup Creek	C	0.42	1.4			0.32	1.1			

Notes: <sup>1</sup> The IDFG license number also serves as the sample ID number.

<sup>2</sup> Before-the-fact treatments were classified before laboratory analysis. The classification was based on distance from a phosphate mine. "M" signified the kill location is in the before-the-fact mine-area and "C" is from the before-the-fact control area.

<sup>3</sup> After-the-fact treatments were classified after laboratory analysis. "E" signified that the liver selenium content is elevated when compared to the control area elk, "C".

Blank cells indicate that there was not value reported.

*Italicized values are less than the UTB for the blank samples*

Table 4.8, *Elk Selenium Data Summary*, presents a summary of selenium data for the Selenium Project elk. The table also includes a summary of liver data from elk harvested elsewhere in Idaho and in Oregon. The selenium concentrations in the Selenium Project elk liver tissue, from both the control and mine areas, are generally within the range of data observed in elk from other parts of Idaho and Oregon. Of the 160 elk livers analyzed, 156 had a liver selenium concentration less than the maximum concentration observed by IDFG in other parts of Idaho (6 to 7 mg/kg [ww]; J. Hansen, IDFG Environmental Staff Biologist, personal communication). The selenium concentrations greater than IDFG's data were 7.4, 7.9, 9.1, and 13 mg/kg (wet), respectively. An evaluation of the Selenium Project elk liver data (on a wet weight basis) from southeast Idaho, other Idaho elk studies (J. Hansen, personal communication), and an Oregon elk study (Stussy, et al., 2000) are presented in Appendix I. Several SeWG agency participants asked if there was a human health concern with the liver that had the 13 mg selenium/kg (wet) concentration. The Selenium Committee is unaware of any selenium tissue concentration benchmarks for protecting human health. To answer this question, a simple and conservative screening assessment is presented in Appendix I demonstrating that consumption of an elk with a 13 mg selenium/kg (wet) liver content is safe a chronic (long-term) consumption basis. The IDHW performed a similar assessment independently and arrived at the same conclusion (E. Shaw-Tulloch, IDHW, Manager, Environmental Health Education Program, Bureau of Environmental Health and Safety, personal communication). To address the safety of short-term exposures, the Selenium Committee conducted a detailed preliminary assessment with input from U of I, IDFG and IDHW. The results indicate a small chance (four percent) of nausea for someone who is:

- successful in killing an elk in the project area;
- a liver eater; and,
- taking selenium supplements.

This assessment shows that the small risk can be abated by either not eating liver or by not taking selenium supplements on the day the liver is eaten.

Dr. Patricia Talcott, a U of I clinical toxicologist, who is assisting the Selenium Committee as a technical expert in veterinary toxicology, performed a cursory evaluation of other trace element (copper, iron, lead, manganese, molybdenum, and zinc) analyses. Dr. Talcott concluded that, with the possible exception of molybdenum, observed concentrations were not indicative of any problems (P. Talcott, personal communication). Dr. Talcott indicated that she could not determine if the molybdenum concentrations are indicative of a problem since there are no diagnostic benchmark tissue concentrations.

The tabular data suggests that there might be a difference between liver and skeletal muscle selenium concentrations from the before-the-fact control- and mine-areas. A principal components analysis (PCA) was performed on the muscle-and-liver data set to allow further visual inspection. A PCA is a data transformation that can be used to take a data set of large dimensions, in this case four dimensions: selenium in muscle, selenium in liver, cadmium in muscle, and cadmium in liver. This generally allows the number of dimensions to be reduced to improve the visual understanding (Pielow, 1994). Plotting the data along the first principal axis, which is defined by tissue selenium content, shows substantial overlap between the 14 before-the-fact control elk and the 77 before-the-fact mine-area elk. This plot is included in Appendix I.

A minimum variance cluster analysis (MVCA) of the muscle-and-liver data indicates the presence of three significant clusters of elk. MVCA is an objective form of data clustering for sample classification (Pielou, 1984). One cluster is a background group of 65 elk (the 14 before-the-fact control elk plus 51 others that cluster with them). The second cluster is a selenium-elevated group of 24 elk. The third cluster is a selenium-elevated-and-low-muscle-cadmium group of 2 elk. Given this information, it appears that there is a difference between the before-the-fact control area and mine-

area elk. The classification of control area elk was redefined, after-the-fact, to contain 65 animals, and the mine-area, or potentially elevated elk, were redefined, after-the-fact, to include 26 animals. This indicates that approximately 29 percent of elk evaluated can be classified in the elevated selenium category. The MVCA and revised PCA plots (using these refined definitions) are also included in Appendix I. The plots can be used to visually confirm the elevated tissue selenium of the group of 26 elevated elk.

The PCA indicates that selenium in liver is the most important variable that can be used to distinguish between the control and elevated elk. Analysis of variance was used to test the hypothesis that selenium in liver alone is sufficient to differentiate these two groups. The null hypothesis was “No,

Statistic	Selenium Project Elk				Other Idaho Elk <sup>1</sup>	Oregon Elk <sup>2</sup>
	Skeletal Muscle Mg/kg (wet)		Liver mg/kg (wet)		Liver mg/kg (wet) <sup>3</sup>	Liver mg/kg (wet) <sup>3</sup>
	Before-the-Fact Control	Before-the-Fact Mine-Area	Before-the-Fact Control	Before-the-Fact Mine-Area		
Range	0.071 – 0.16	0.060 – 0.93	0.28 – 0.89	0.21 - 13		
Sample Size	14	77	14	77	5 – 90	26 – 71
Mean	0.12	0.22	0.47	1.6	0.27 – 1.29	0.080 – 1.78
Standard Deviation	0.029	0.14	0.19	2.1	0.34 – 1.67	0.04 – 1.06
Standard Error	0.0076	0.015	0.050	0.22	0.074 – 0.46	0.006 – 0.20
Notes: <sup>1</sup> These data are from IDFG studies from four non-phosphate mine areas (Hanson, personal communication). Data are summarized in Appendix I.						
<sup>2</sup> These data are from three non-phosphate mine areas (Stussy et al., date unknown). Data are summarized in Appendix I.						
<sup>3</sup> We have assumed the results are on a wet-weight basis.						

selenium in liver alone can not be used to differentiate,” versus the alternative hypothesis of “Yes, selenium in liver alone is sufficient to differentiate.” The result (presented in Appendix I) is highly significant ( $p < 0.050$ ), and the null hypothesis is rejected and we now hypothesize that selenium in liver alone can be used to differentiate between control elk and elevated elk. Appendix I contains a plot of selenium liver content versus kill site distance from a phosphate mine. Although there is substantial scatter, an obvious inverse relationship is evident, the closer the kill site to a mine, generally the higher the liver selenium concentration.

Correlations between the various elk investigation variables are also tabulated in Appendix I. The results indicated that selenium concentrations in liver and muscle are significantly and positively correlated with one another, and both are significantly and negatively correlated with kill-site distance. These are the only significant correlations. (The correlation table provides correlations between certain variables that are shaded). These are provided for information only, not for hypothesis testing. For example, correlations with for the after-the-fact classification is artificial, because the classification effort was undertaken in the hopes of finding a significant correlation.

However, the results indicate that the effort was successful. Correlations with principal components scores are redundant, given that PCA is successful only if there are significant correlations. However, the results confirm, for example, that the first principal axis, which contains 55 percent of the information in the data set, can be defined as a selenium and inverse kill-site distance axis. Correlating principal components scores amongst themselves is also redundant, because principal axes are, by definition, uncorrelated with one another.

Cadmium data for the southeast Idaho elk are also presented in Table 4.7. The tabulated data suggest that there are no differences in cadmium tissue concentrations between after-the-fact control area and

mine-area elk. Analysis of variances presented in Appendix I confirm this ( $p \geq 0.050$ ). Given that there is no significant correlation between cadmium concentrations in the two tissues, that cadmium concentrations are not correlated with selenium concentrations or kill-site distance, and that it is reasonable to hypothesize that phosphate mining is associated with elevated selenium tissue concentrations, it appears that phosphate mining is not affecting elk tissue cadmium concentrations in any way.

### 4.3.3 Cattle Investigation

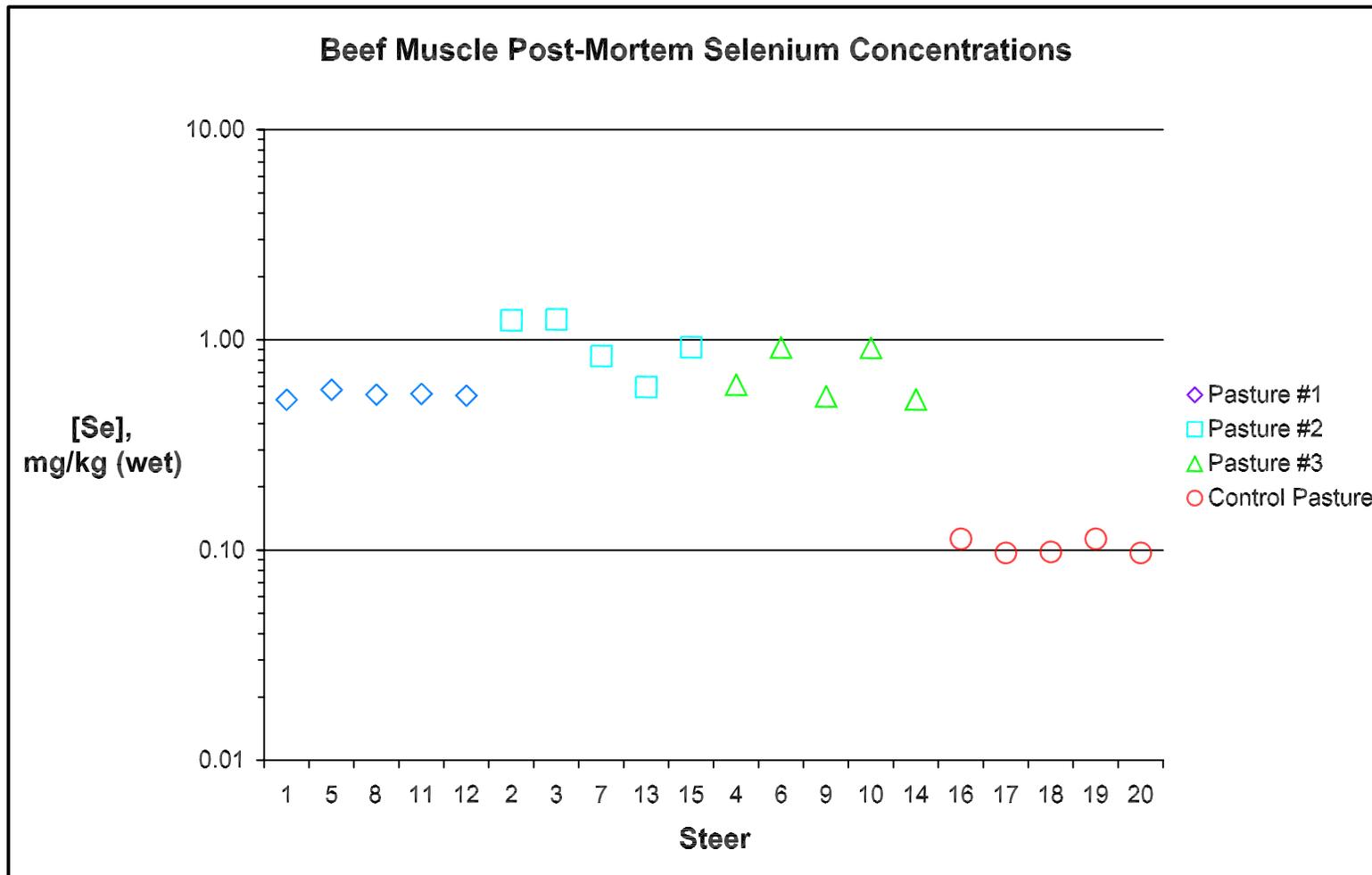
Twenty steers were delivered to the U of I for a feedlot depuration study on October 1, 1999. Fifteen of the steers were pastured on a Henry Mine reclaimed overburden dump for nine weeks in July and August. The remaining five steers, which were from the same herd, were grazed in the vicinity of Grays Lake Wildlife Refuge. Prior to transport to the U of I, all the animals were grazed on non-seleniferous forage for 30 days. This is typical of normal cattle handling practices in the Soda Springs area, where cattle are grazed on lowland pasture prior to shipment to a feedlot.

Upon arrival at the U of I, the steers were allowed to acclimate for seven days before tissue and blood samples were collected and feedlot rations began. The depuration study lasted for approximately 120-days. The steers were fed a diet containing 0.3 mg selenium/kg (wet, but air-dried; the maximum concentration of selenium allowed under feedlot regulations).

Samples were analyzed for the parameters presented in Table 3.5. Liver and skeletal muscle tissue biopsy samples were collected three times; at approximately day-104, -147 and -197 of the study. Whole blood and serum was sampled every two weeks. The tissue biopsy, blood and serum data will be released under separate cover by Dr. Jim Kingery, the studies principal researcher and Mr. Jeff Knight, the research assistant.

Five steers each were slaughtered on February 7, 9, 22 and 23, 2000. Post-mortem skeletal muscle, liver, kidney and heart tissue were sampled and analyzed at the U of I's Animal Sciences Laboratory. Appendix J includes the post-mortem sample laboratory results. Table 4.9, *Post-Mortem Beef Tissue Selenium Laboratory Data*, presents selenium concentrations measured in steer skeletal muscle, liver, kidney, and heart at the time of slaughter. All five control steers had muscle selenium content less than 0.50 mg/kg (wet). This is within the range that is considered non-toxic from the perspective of animal health (Puls, 1994). Two animals had muscle content greater than 1.2 mg selenium/kg (wet), which is greater than an Australian standard for human consumption of beef skeletal muscle. Table 4.10, *Summary of Post-Mortem Muscle Selenium Content*, summarizes the selenium concentration in post-mortem muscle tissue by pasture.

Drawing 4-6, *Beef Muscle Post-Mortem Selenium Concentrations*, presents a graphical display of the data by pasture. The steers that were grazed on the Henry Mine pasture had selenium muscle content in the post-mortem samples that were significantly higher than the five control steers. While the steers that were grazed on seleniferous forage had muscle selenium content that was higher than in the control steers, the observed selenium concentrations are well within the range of 0.02 mg selenium (wet)/kg to 3.0 mg selenium/kg (wet) for beef cattle skeletal muscle reported by Ihnat (1989). Moxon et al. (1994) reported that the high end of Ihnat's range comes from cattle raised in South Dakota on a naturally seleniferous diet.



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 <b>MONTGOMERY WATSON</b>			PROJECT No.: 1227028.021805 AutoCAD FILE: 1999INTERM.FIG4-6 SCALE: N.A.      FIGURE No: <b>4-6</b>		

*IDAHO MINING ASSOCIATION  
SELENIUM COMMITTEE*

**BEEF MUSCLE POST-MORTEM  
SELENIUM CONCENTRATIONS**

Steer	Treatment	Skeletal Muscle mg/kg (wet)	Liver mg/kg (wet)	Kidney mg/kg (wet)	Heart mg/kg (wet)
1	Pasture #1	0.52	0.70	1.2	0.44
5	Pasture #1	0.58	0.60	1.5	0.47
8	Pasture #1	0.55	0.63	1.3	0.45
11	Pasture #1	0.55	0.49	1.6	0.45
12	Pasture #1	0.54	0.51	1.5	0.46
2	Pasture #2	1.2	0.85	2.2	0.67
3	Pasture #2	1.3	0.83	1.6	0.67
7	Pasture #2	0.84	0.84	1.6	0.55
13	Pasture #2	0.60	0.66	1.4	0.56
15	Pasture #2	0.92	0.60	1.5	0.53
4	Pasture #3	0.61	0.83	1.8	0.56
6	Pasture #3	0.92	0.91	1.7	0.58
9	Pasture #3	0.54	0.82	1.6	0.59
10	Pasture #3	0.92	0.54	1.8	0.49
14	Pasture #3	0.52	0.54	1.8	0.54
16	Control	0.11	0.44	1.1	0.24
17	Control	0.10	0.35	1.2	0.25
18	Control	0.10	0.41	1.4	0.27
19	Control	0.11	0.46	0.93	0.26
20	Control	0.10	0.45	1.6	0.26

Steer ID	Pasture	Range of Selenium Concentration mg/kg (wet)	Sample Mean Selenium Concentration <sup>1</sup> mg/kg (wet)	Estimated Standard Deviation Selenium Concentration <sup>2</sup> mg/kg (wet)
1,5,8,11, and 12	No. 1	0.52 – 0.54	0.55	0.023
2, 3, 7, 13, and 15	No. 2	0.60 – 1.2	0.97	0.32
4, 6, 9, 10, and 14	No. 3	0.52 – 0.92	0.70	0.21
16, 17, 18, 19, and 20	Control	0.10 – 0.11	0.10	0.0090

Notes: <sup>1</sup>Minimum variance unbiased estimate assuming 2-parameter lognormality.  
<sup>2</sup>Minimum variance unbiased estimate assuming 2-parameter lognormality.

The United States Department of Agriculture's Food Safety and Inspection Service (FSIS) requested selenium content data in skeletal muscle from the 20 steers involved in the Selenium Project study. The FSIS requested the information because of potential human health risks from eating beef with high selenium residue in the muscle tissue. A report summarizing the study post-mortem results was prepared and transmitted to the FSIS on March 21, 2000. This report is presented in Appendix K. As part of the FSIS report, the Selenium Committee prepared a simple conservative bounding estimate of risk to demonstrate that the beef is safe to eat on a chronic-consumption basis. The FSIS, in consultation with Food and Drug Administration (FDA), determined that the two steers with skeletal muscle content of 1.2 mg selenium/kg were unfit for human consumption. FSIS personnel subsequently destroyed these two carcasses that were being held under standard chain-of-custody at a Moscow, Idaho food locker.

#### 4.3.4 Cutthroat Trout Investigation

There were three components to the cutthroat trout investigation. One is an egg-viability study to assess if observed selenium concentrations are causing birth defects in this native indicator species. The second is a feeding trial to assess if elevated dietary selenium impacts growth rates, survivorship, or subsequent breeding success in native fish. The third is a genetic analysis to evaluate whether selenium exposures might impart a survivorship bias on the results of the previous two studies.

Blackfoot River eggs for the viability study were obtained by electrofishing and stripping eggs from ripe females. At the same, a small piece of adipose fin was clipped from these ripe females and other adult fish and the fin was stored in 70% ethanol until the genetics analysis was performed. Control eggs were obtained from ripe females returning to a state hatchery on Henry's Lake. The feeding trial is being performed on the control and Blackfoot River progeny from the egg-viability study.

#### 4.3.4.1 Egg-Viability Study

IDFG and Hagerman Experiment Station personnel collected mature cutthroat trout from the Blackfoot River by electrofishing near stream monitoring station ST026 on June 9, 1999. Ripe females were stripped of their eggs, and the eggs were stored in an iced cooler until they were transported to the Hagerman Experiment Station. Ripe males were stripped to collect milt, which was placed into individually labeled cups, and stored in an iced cooler.

Standard Hagerman Experiment Station procedures were used for fertilization and incubation. Details are presented in the study progress report, which is included in Appendix L. Dead and deformed fish were removed and counted at the initial pick-off stage, eyed stage, and at the time of hatching. Samples of eyed eggs, yolk-sac fry, and swim-up fry were removed for laboratory analysis of whole body selenium.

Control eggs at the eyed stage were obtained from the IDFG Henry's Lake hatchery on May 6, 1999. These eggs were incubated in the same manner as the Blackfoot River cutthroat trout eggs. Dead and deformed fish were removed and counted, and samples of eyed eggs and yolk-sac fry were analyzed for whole body selenium.

#### Blackfoot River Cutthroat:

A total of 93 cutthroat trout were collected from the Blackfoot River on June 9, 1999. Thirty-six adult females and four mature males were identified. Of these, only four females were spawned and two ripe males found. Of the four females, two had recently spawned, but still had a small number of ripe eggs (about 300 eggs). Two ripe females were ready to spawn. One of these latter females produced about 1,700 eggs, and the second produced about 1,800 eggs. Eggs from the two fully-spawned females were incubated in separate trays, while the eggs from the two-partially spawned females were combined into one tray. Table 4.11, *Cutthroat Trout Egg-Viability Study Results*, summarizes egg hatching success, number of deformed fry, and egg selenium content.

Female <sup>1</sup>	No. Eggs Collected	Hatching Success (percent)	Deformed Fry		Selenium Content Mg/kg (dry)	
			No.	Percent <sup>2</sup>	Eyed-egg	Yolk-sac
No. 1	~1,700	98	4	0.7	4.4	1.4
No. 2	~1,800	99	11		6.7	2.1
Nos. 3 and 4	~300	98	13		4.0	
Henry's Lake	~3,200	98	19	0.6	1.4	1.6

Notes: <sup>1</sup> Eggs from females 3 and 4 were pooled together into one sample because of the limited number of eggs.  
<sup>2</sup> Total percent for all Blackfoot River fry.

#### Henry's Lake Cutthroat:

These eggs were obtained as eyed eggs, consequently, there was no opportunity to measure percent fertility. Table 4.11 also includes information on the Henry's Lake eggs.

The data indicate that the selenium concentrations in Blackfoot River eggs were higher than the Henry's Lake eggs. However, the percentage of deformed fry was similar and was an order of magnitude below the percentage, six percent, described by Lemly (1993) as being the upper range of background for fish not exposed to elevated levels of selenium. Assuming that the percentage of deformities observed in the three batches of Blackfoot River eggs are lognormally distributed, the one-sided upper confidence limit of the mean deformity rate in Blackfoot River fish, as determined by simulation, is 4.0 percent.

These results indicate that, while the Blackfoot River cutthroat trout eggs have three-to-five times more selenium than do the control eggs, there is no discernable difference in the rate of birth defects. A selenium toxicity reference benchmark for increased birth defects in fish of 10 mg/kg (dry) in eggs or ovaries has been suggested (Lemly, 1993). The selenium levels of all three batches of Blackfoot River eggs, although elevated above the control concentration, are well below this benchmark. Furthermore, it appears that the tabulated egg concentration data are not correlated to the birth defects rate data. These observations suggest that the exposures to elevated water-column selenium are insufficient to cause toxic accumulation in eggs, or that cutthroat trout may not accumulate selenium as readily as some other species. If the former is the reason, limited exposure could result from natural fish behavior, adapted fish behavior, the limited duration of the spring pulse of selenium in adult fish habitat, fish physiology, or a combination of these factors.

Because the number of ripe females obtained from the Blackfoot River was much lower than desired, the IMA Selenium Committee is regarding these results as preliminary. Another collection effort will be undertaken in spring 2000 (earlier in the spawning season) to obtain a larger sample size of Blackfoot River fish, and fish of an appropriate control watershed, and the study will be repeated.

#### **4.3.4.2 Feeding Trial**

A feeding trial is being conducted to determine the effects of various dietary concentrations of selenium, added as selenomethionine, of feed intake, growth, and reproductive performance of cutthroat trout. The following discussion summarizes the results of the first year of the study. A detailed progress report on the feeding trial is included in Appendix L.

##### Henry's Lake Cutthroat:

When the fish were one (1) gram in average weight, they were randomly selected and grouped into groups of 20 fish, weighed and placed into 18 50-liter tanks. During the first several weeks the fish were fed ten times per day, six days per week to apparent satiation. Feeding frequency decreased as the fish grew. Each dietary treatment was fed to triplicate groups of fish, positions at random throughout the rearing system.

Six experimental diets were prepared. The diets were similar to commercial trout diets with the exception of amount of selenomethionine added to each diet. Two samples of the experimental diets were analyzed. Results of the first samples showed that diet 1 (no selenomethionine supplement) had a selenium concentration in the diet was 1.4 mg/kg dry feed weight. The remaining experimental diets contained 4.1, 6.4, 10.0, 12.0, and 12.0 mg selenium/kg dry feed weight. The second diet sample yielded a level of 0.94 mg/kg dry feed in the control diet, and 3.41, 5.48, 7.82, 8.71, and 10.3 mg selenium/kg dry feed for the five diets to which selenomethionine was added. The expected selenium concentrations would have been 1.4, 3.4, 5.4, 7.4, 9.4 and 11.4 mg/kg dry feed for the six diets. Differences between analyzed and expected values are likely the result of variation in the selenium concentration of the small amount of feed that was actually analyzed.

Fish in each tank were bulk-weighed and counted every 14 days for the first 12 weeks. Thereafter, the fish were weighed and counted every month. Dead fish were removed and counted daily. Fish

samples for whole body selenium analysis were collected at each sampling date for the first 12 weeks from the initial population and from two of three replicate tanks for each dietary treatment group. Thereafter, the fish were sampled at three month intervals. Selenium content is reported on a wet-weight basis.

#### Blackfoot River Cutthroat:

When the fish were 0.1 gram in average weight, they were randomly selected and grouped into groups of 75 fish, weighed and placed into 21 150-liter tanks. During the first several weeks the fish were fed ten times per day, six days per week to apparent satiation. Feeding frequency decreased as the fish grew. Each dietary treatment was fed to triplicate groups of fish, positions at random throughout the rearing system.

The same diets that were prepared for the Henry's Lake cutthroat trout were used in the feeding trial of the Blackfoot River cutthroat. Diets were assigned to replicate tanks of fish in a completely randomized design. Each experimental diet was fed to triplicate tanks of fish.

#### Interim Results and Discussion:

The Henry's Lake fish were started in the feeding trial on June 23, 1999. The average weight was 0.9 grams. By the end of March, 2000, the fish weighed an average of 140 grams (treatment range: 132 – 146 grams) and appeared to be growing normally. There was no relationship or trend between dietary treatment and average fish weight. Whole body selenium content differed among dietary treatment groups, ranging from 0.4 mg selenium/kg to 2.9 mg selenium/kg, in samples taken after 26 weeks of feeding (January 31, 2000). Mortality has been negligible in the Henry's Lake cutthroat trout throughout the feeding trial and does not appear to be associated with dietary treatment.

The scientific literature suggests that selenium toxicity in salmonids is reached at a specific body level, which Hamilton et al. (1990) reports to be 4.5-5.0 mg selenium/kg (dry weight) in chinook salmon fry. The Henry's Lake post-juveniles have reached twice this body level, over a six month period, without showing clinical signs of toxicity. The Henry's Lake cutthroat trout will continue to be monitored for feed intake to detect any change associated with dietary treatment, and to sample the fish quarterly for whole body selenium content.

The Blackfoot River cutthroat were started in the feeding trial on August 1, 1999, at an average weight of 0.1 grams. Problems were observed within two weeks of initiating the feeding trial. Persistent, low-grade mortality ensued, more-or-less equal across dietary treatment groups. Clinical signs resembling pyridoxine (vitamin B<sub>6</sub>) deficiency were noted, and supplementation of the diets with fresh beef liver resolved the mortality problem within three days. However, weight gain was non-existent and a generalized wasting was evident in all tanks. No diet formulation developed for rainbow trout, open-formula, experimental, or commercially available, was accepted by the fish. Consequently, these fish were removed from the feeding trial. Discussions with state and federal hatchery personnel at other locations where wild cutthroat trout were raised revealed similar observations at all stations when fed any commercial diet or agency-specified diet. A completely new diet formulation was developed at the Hagerman Experiment Station, tested for eight weeks, and tested informally at a USFWS cutthroat trout hatchery where cutthroat trout of wild origin were being raised. Results have been extremely positive. The feeding trial with the Blackfoot River cutthroat has been re-started using the new diet formulation as the base to which selenomethionine is added.

At present growth rates, cutthroat trout from both groups are expected to reach first spawning in approximately one year, at which time the effects of dietary treatment on reproductive performance will be assessed. In the meantime, the fish will be carefully observed for any overt signs of selenium toxicity.

#### **4.3.4.3 Genetic Analysis of Blackfoot River Cutthroat Trout**

A genetic analysis of Blackfoot River cutthroat trout was conducted by Dr. Matt Powell from the Hagerman Experiment Station. The three objectives of the study were:

- 1) To determine the taxonomic status of cutthroat trout samples collected from the Blackfoot River and Henry's Lake Hatchery in 1999.
- 2) To determine if cutthroat trout collected from Henry's Lake, Idaho, being used in selenium diet experiments, are genetically different from those collected from the Blackfoot River.
- 3) To determine if cutthroat trout from the Blackfoot River watershed lack genetic variation as compared to cutthroat populations from adjacent areas.

The management and conservation of cutthroat trout have become priorities for several state and federal agencies due to the decline of Yellowstone cutthroat trout populations throughout their historic native range. In August 1998, the USFWS was petitioned to list the Yellowstone cutthroat trout as a threatened species under the Endangered Species Act. Currently, the IDFG recognizes all cutthroat trout as a "species of special concern".

Fin samples were collected from Henry's Lake, Willow Creek, and Blackfoot River cutthroat trout and stored in 70 percent ethanol or preservation/lysis buffer until DNA was extracted using methods modified from Sambrook et al. (1990) and Dowling et al. (1990). Total genomic DNA was isolated from each sample. Details of the DNA testing are included in Appendix L.

The mitochondrial genetic analysis confirmed the samples collected from the upper Blackfoot River and Henry's Lake are Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*). No evidence of rainbow trout introgression was observed in any samples from either population.

On the basis of both mitochondrial DNA and nuclear DNA (nDNA) analyses, the population of Yellowstone cutthroat trout in the upper Blackfoot River is different from the population of Yellowstone cutthroat trout in Henry's Lake. Because they are the same subspecies, the Henry's Lake cutthroat are likely good controls and surrogates for the Blackfoot River fish. However, an alternative control population, from Willow Creek (a Snake River tributary in close geographic proximity to the Blackfoot River), is much more closely related to the Blackfoot River population, showing no evidence of difference on the basis of nDNA analysis. Another advantage of using Willow Creek fish in future studies as controls is that they, like the Blackfoot River cutthroat, are wild, unlike the hatchery raised fish from Henry's Lake.

There is no evidence to indicate that the population of Yellowstone cutthroat trout in the Blackfoot River have less genetic diversity than what is observed in Yellowstone cutthroat trout populations that do not dwell in seleniferous habitats. Thus, there is no evidence that empirical investigations of the Blackfoot River population of Yellowstone cutthroat trout will be affected by survivorship bias.

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## **Section 5**

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## 5.0 REFERENCES

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# IDAHO MINING ASSOCIATION

## SELENIUM COMMITTEE

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### 1999 Interim Investigation Data Report

Southeast Idaho  
Phosphate Resource Area  
Selenium Project

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October 2000

*Prepared by:*



**MONTGOMERY WATSON**

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## Appendix A

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# **Field Manual**

## **Analysis of Selenium and Cadmium Levels in the Eggs of Selected Avian Species (Revision 0, July 1999)**

**Southeast Idaho Phosphate Resource Area - Selenium Project**

*Spring and Summer 1999*

*Prepared by:*

**John T. Ratti, Research Professor**  
**Department of Fish and Wildlife Resources**  
University of Idaho  
Moscow, Idaho 83844-1136

## INTRODUCTION

Five Companies, or their predecessors, have operated or are currently operating phosphate mines in the Southeastern Idaho Phosphate Resource Area. As with most environmental perturbations, mining has resulted in the production of a number of by-products that represent potential environmental contaminants. These include trace elements such as selenium and cadmium.

In 1996 a rancher near Soda Springs reported that 6 horses were sick, and subsequent tests conducted at the University of Idaho revealed chronic selenosis (selenium poisoning). Consequently, a number of studies have been initiated to determine the level of selenium (and other trace elements) in the environment as a direct result of mining activity. This study will be focused only on potential impacts to bird populations. Trace elements of selenium and cadmium will be measured from tissues in avian eggs as an index to exposure.

## OVERALL STUDY DESIGN

This study is designed for a minimum of 2 years of field-data collection during the spring and early summer of 1999 and 2000. The primary objectives are to obtain an appropriate sample of avian eggs to determine:

- 1) if selenium and cadmium contaminants in watersheds associated with mining activity are present in avian tissues (eggs), and
- 2) if present, are selenium and cadmium levels significantly different from background (i.e., control) areas in the immediate region not disturbed by mining.

For both mining and background areas, 4 distinct habitats (strata) will be sampled:

- 1) tributary streams (small, generally 1<sup>st</sup>-or 2<sup>nd</sup>-order streams);
- 2) ponds and wetlands;
- 3) rivers; and,
- 4) lakes and reservoirs.

Within the impact areas, these habitats are represented by ponds near mining sites, the Blackfoot River (which is fed by numerous 1<sup>st</sup> order streams in the impact zone), and Blackfoot Reservoir (which is fed by streams and the Blackfoot River).

The selection of avian species for inclusion in this study will be limited by the species approved for egg collection by the U.S. Fish and Wildlife Service (USFWS) collecting permit (and similar restrictions that may be imposed by the Idaho Department of Fish and Game [IDFG], Grays Lake National Wildlife Refuge [NWR], land owners such as the Bureau of Indian Affairs and Shosone-Bannock Tribes, etc.). A list of all species approved for egg collection will be provided to each field research assistant. A **tentative** list submitted by the USFWS, IDFG and Grays Lake NWR is included as Attachment 1. Given the above restrictions, the study researchers will attempt to collect eggs from at least 3 species in each of 3 trophic groups (i.e., herbivores, omnivores, and carnivores) from each of the 4 strata. The statistical goal is to collect a minimum of 5 eggs from each species within each trophic group for each strata. The overall goal for the 2-year study effort is to collect 360 eggs. Table B-1 presents the collection matrix (72 cells X 5 eggs/cell = 360 eggs).

**TABLE B-1  
PROPOSED BIRD EGG COLLECTION DESIGN**

<b>Treatment Area – Mining Impacts</b>					
<b>TROPHIC REPRESENTATION</b>		<b>Strata</b>			
		<b>Tributary Streams</b>	<b>Ponds Wetlands</b>	<b>River</b>	<b>Lakes and Reservoirs</b>
Herbivores	Spp One	5	5	5	5
	Spp Two	5	5	5	5
	Spp Three	5	5	5	5
Omnivores	Spp One	5	5	5	5
	Spp Two	5	5	5	5
	Spp Three	5	5	5	5
Carnivores	Spp One	5	5	5	5
	Spp Two	5	5	5	5
	Spp Three	5	5	5	5
<b>Totals</b>		<b>45</b>	<b>45</b>	<b>45</b>	<b>45</b>
Note: Soil samples will be collected at each sampling unit.					
<b>Control Area -- Background</b>					
Herbivores	Spp One	5	5	5	5
	Spp Two	5	5	5	5
	Spp Three	5	5	5	5
Omnivores	Spp One	5	5	5	5
	Spp Two	5	5	5	5
	Spp Three	5	5	5	5
Carnivores	Spp One	5	5	5	5
	Spp Two	5	5	5	5
	Spp Three	5	5	5	5
<b>Totals</b>		<b>45</b>	<b>45</b>	<b>45</b>	<b>45</b>
Note: Soil samples will be collected at each sampling unit.					

Obtaining adequate data for each cell may be difficult, i.e., actual egg collections may be limited by collecting permits and permission, the numbers of species, the abundance of each species, and variation in the difficulty of locating nests among species. Trophic representation will be determined after the field season is complete, which may also place limitation of the research design. Thus, we anticipate some adjustment to the egg-collection design after the first year, i.e., we may limit our year-2000 collection to those species found to be most abundant, yet available in all strata and representative of the 3 trophic groups. This potential methodology shift will allow us to focus on specific species in specific strata and, therefore, maximize efficiency of field work in 2000 (i.e., we will not waste time searching for species with low probability of finding adequate samples to meet requirements of statistical analysis).

A stratified-random sampling approach will be used for egg collection. For tributary streams and rivers, suitable study areas will be mapped and segmented in to numbered 0.5 mile linear units of riparian habitat. An equal number of 0.5-mile study units will be selected randomly for both mining sites and background sites. For ponds and reservoirs, potential study sites will be mapped and estimated area of habitat adjacent to the water bodies will be determined (e.g., areas of islands, and the area of riparian habitat surrounding the periphery of ponds and lakes). These areas will be broken into square mile segments and numbered. Sampling units will consist of randomly selected square-mile blocks, or portions thereof (e.g., ¼ segments). The study will attempt to match our non-control sites with previously

selected sites used for sediment sampling, especially those sites that were selected with a randomized design (Bill Wright, Montgomery Watson, personal communication).

Colonial nesting species (e.g., gulls, ibis, cliff swallows) present a special sampling problem in that their nests are grouped at a few isolated patches. For such species we will locate as many colonies as possible and randomly select colonies for egg collection, and randomly select nests within colonies.

Dr. John Ratti and Mr. Dan Golner established research sites during the week of May 10<sup>th</sup>. Each field research assistant will be supplied with a map of the “search areas” for each strata in both mining and background areas.

## **THE RESEARCH TEAM**

John Ratti (Research Professor, University of Idaho) is the Principal Investigator for this project. Research Assistants include Dan Golner (University of Wisconsin, Stevens Point), Emily Drew (Western Washington University), Michael Lemoine (University of Idaho), Curt Mykut (Goucher College), and Matthew Wilson (Utah State University). All members of the research team have a college degree in wildlife biology (or a closely related field) and experience with avian field studies. Dan Golner will be the on-site research team supervisor. Dan has both B.S. and M.S. degrees in Wildlife Science, and experience on numerous field projects in several states. Dan has worked for Ratti as a Research Assistant for the past 3 years.

## **LAND ACCESS**

Nearly all of our nest-searching activities will be on private lands, or lands administered and controlled by Gray’s Lake National Wildlife Refuge, Bureau of Indian Affairs, Bureau of Land Management, and the U.S. Forest Service. We must respect all laws and regulations regarding access to such lands. In many cases, we will need special permission to conduct our work (Dan Golner and John Ratti will be responsible for such communications). We need to be especially careful with our work on Gray’s Lake National Wildlife Refuge. The refuge has several sensitive species nesting and they also have ongoing research activities. Thus, we must coordinate all work on refuge lands with Wildlife Biologist Bill Pyle (208-574-2755).

## **NEST SEARCHING**

Each research assistant will be provided with a list of species included on our collecting permit. This list will contain less than 30 species. For each species that you are not completely familiar with, you will be responsible for reading about behavior, habitat, and nest structure and location. Ratti will supply Dan Golner with several resource books for your research. Also, for species and nests where you have little or no experience, be sure to discuss these with other member of the Research Team, so you can learn from their experiences. Thus, for each species on the collecting permit list, you will be responsible for identification, song and call notes, nest locations, and general nest structure (you will be provided with CD player and a CD of bird songs and calls for all avian species in Idaho).

Nest searching methods will vary considerably based on circumstances and target species. Nests for some species such as Canada goose, American coot, Franklin’s gull, and barn swallow are relatively easy to locate, i.e., if the observer is near the nest site, he/she will likely find the nest if you look to characteristic locations and note protests from attending birds.

Nesting territories of some other species are easy to locate by protests of attending birds (e.g., killdeer), but often locating the actual nest may require patient observation. For species such as killdeer the best approach is to quietly move away from the area that solicits the greatest behavioral response from the birds (e.g., wing feigning), and to sit quietly and watch for the bird to return to the nest site. Your observation point must be far enough away that the bird again feels secure in returning to the nest (i.e., the predator is no longer a threat), but close enough that you can observe the bird). Sometimes this can be accomplished at a fairly close distance if you are able to hide behind some type of cover. In other cases with very open habitat, you may have to move a long distance away and watch very carefully. For shy species you may need to use a spotting scope to actually see the bird return to the nest site. For some precocial species you may spend a fair amount of time waiting to locate a nest site, only to discover that the neonates have already hatched and left the nest (i.e., the alarm calling was associated with protection of free-ranging young birds, not the nest site).

For other species, especially many Passerines such as horned lark and song sparrow, the process of finding nest can be difficult. This is particularly true during incubation, when we need to find nests. Nests of many Passerine species are obvious during frequent feeding trips to the nests after eggs hatch, but during incubation they are secretive and less aggressive in defending territories. Often nests for such species are located only from patient movement through potential nesting habitat and watching for birds flushing from the nest, signs such as subtle alarm calls, or movement back to the nest. Nearly all species take a break from incubation to feed and preen, and these movements provide good opportunities to locate nest sites. For most species, these incubation breaks are during early morning and late afternoon.

For this study, one important nest-location method will be to watch for birds carrying nesting material. Some species (e.g., killdeer) do not use nesting material and simply lay eggs on the ground among cryptically colored rocks. However, most species gather some form of nesting material. Cliff and barn swallows gather mud from puddles or the edge of permanent water bodies; robins and house wrens build nests from grasses, leaves, feathers, string, etc. Nest-building activities (i.e., movements to the nest site with nesting material in the mandible) offer the easiest opportunity to find nests, especially nests of certain species that hide the nest bowl well, and sometimes in difficult-to-see locations.

## MARKING NEST SITES AND DISTURBANCE FACTORS

Nest sites will be recorded in different manners based on the stage of the nesting cycle:

- 1) nests under construction;
- 2) nests with eggs;
- 3) nests with neonates that have not fledged; or,
- 4) nests that have hatched eggs but the young are absent (this latter case would include altricial neonates that have successfully fledged among species, neonates that may have been depredated, and precocial neonates that have left the nest immediately after hatching).

For nests under construction or with eggs, move away (approximately 50-150 feet depending on cover conditions) from the nest site immediately to lessen disturbance at the site and to minimize the possibility that the bird in question will abandon the nest site. It is also important not to remain near the nest site for any period of time because many mammalian predators seem to have developed a search image associated with investigating the trailing odor of other organisms such as man ... i.e., man's activities near nest sites seem to have increased predation rates from some predators such as skunk and raccoon. With your GPS unit, record the latitude and longitude of your location some distance **AWAY FROM THE NEST**. Then record a compass direction **AND** distance (estimated in feet) to the nest site. Place a marking flag at your GPS location (not the nest location), and write a description of the nest-site location the location of your marking tape. Also write a general description of the nest location, e.g., "in a willow

tree 130 meters downstream from the Dry Creek bridge.” The bottom line here is that if we need to return to the nest site, there should be **NO CONFUSION** regarding the general or specific location.

When you locate a nest with neonates, an abandoned nest with eggs, or an empty nest that you suspect is from the 1999 season (and you think you can identify the species), we will also record nest-location data. Since we are only interested in egg collection, these nests will not provide data for 1999, but recording the nest location may help us for data collection in the year 2000 (i.e., most avian species are somewhat philopatric and exhibit nest-site homing behavior ... “the act of returning to an original location”). For nests with neonates in the nest, it is especially important to quickly move from the area (as described above) before recording the nest site. Although disturbance will seldom cause abandonment of nests with neonates, these nests are more vulnerable to predation. For empty or abandoned nests, you may record nest-location data from the immediate nest site (however, be cautious not to quickly judge a nest as abandoned).

Dr. Ratti and Dan Golner will establish artificial nest sites with chicken eggs for a training session on recording nest data the first week of field work.

## **EGG COLLECTION, STORAGE, AND SHIPMENT**

Before any egg is collected, you must have a positive identification to species level. In all cases you will be expected to accomplish this by observation of a bird at the nest site, or by obvious alarm calling (and other behaviors) of a bird at the nest site. However, you are cautioned that on some occasions multiple species will alarm call in response to a potential predator (i.e., sometimes several birds of several species will assist in harassment of a potential predator). In such cases you may need to leave that area and return the next day to reconfirm species identification.

Eggs will be numbered with a fine-point marker (if necessary). Numbering will start with the egg in the most northerly position of the nest bowl, and will proceed in a clockwise direction, and then toward the center. With nests having fewer than 6-8 eggs, this process can be conducted mentally without marking the eggs. However, some nests may have too many eggs for a mental tally, and marking will be necessary. For nests with a cavity structure (e.g., flicker or cliff swallow), it will not be feasible to count and number eggs. For these exceptions, we will simply reach into the nest cavity with a “mechanics fingers” tool to take an egg. Since you will not be able to see the eggs, no observer bias is likely and we can assume such egg-collection methods will be random. For cowbirds, often there will be only one parasitic egg in the host nest, and this egg may be collected without concern for a random-selection process.

For communal nesting species, you will map nests from an arbitrary location within the colony, number the nests on your map (within view from one spot), and then determine nests for egg collection with the use of random numbers.

One egg will be taken from each nest according to a systematic rotational sequence (you will be provided with data sheets that have a numbering system). Only one egg will be collected from each nest in order to maintain statistical independence among samples, and in accordance with the collecting permit.

Each egg collected will be assigned a unique alpha-numeric code (to distinguish from the random-selection numbering), which will match with a data form. Instructions for the alpha-numeric code will be described on the data sheet. Eggs will be marked with waterproof ink pens (e.g., Sharpie®). On the data form we will record the species, clutch size, latitude and longitude of the recording site near the nest,

written description of the nest location, date, time, and name of the person collecting the egg. Protocol for egg storage and shipment will be consistent with standard-field-sampling techniques. Each egg will be washed with water, rinsed with distilled water, air dried for several minutes, placed in a heavy-duty zip-lock bag, and stored in a padded container. After an egg is collected in the field, return to your vehicle immediately and place the eggs in the thermoelectric cooler. At the end of the day, eggs will be transferred to a conventional refrigerator for storage at  $<4^{\circ}\text{C}$ . Eggs will be shipped with blue ice every 10 days (more frequently if necessary due to many eggs in storage) via Federal Express directly to:

**Tom Case**  
**Analytical Sciences Laboratory, Holm Research Center**  
**University of Idaho, Moscow, ID 83844-2201**  
**(208) 885-7081**

Eggs may be shipped **ONLY** Monday through Thursday (i.e., no Friday shipments because of delivery problems on weekends). On the day that eggs are shipped, a call must be placed to the Laboratory (208-885-7081) to inform someone that eggs are being shipped and to anticipate delivery the next day. Do not ship the eggs unless you are successful at delivery of this telephone message. Dan Golner will be responsible for egg shipment (however, Dan may instruct other members of the team to complete this task if necessary).

## **LABORATORY ANALYSIS**

The bird eggs will be analyzed for selenium and cadmium concentrations following analytical protocols developed by the University of Idaho's Analytical Sciences Laboratory. These protocols were developed in consultation with USFWS researchers. The following discussion summarizes the Analytical Sciences Laboratory protocol.

- Eggs will be stored in the laboratory at  $4^{\circ}\text{C}$  until they are to be analyzed.
- The egg morphometry will be determined by measuring length, width, weight and volume. The volume will be determined by displacement in water if the shell is not cracked. If the shell is cracked, volume will be determined by a species-specific calculation.
- The egg will be evaluated for reproductive effects. First, one end of the egg will be opened carefully with a forceps to check to see if the embryo is in the correct position. If not, this may be a sign of adverse impact. Next, the egg will be broken and emptied into a clean, sterilized Petri dish to look for deformities.
- To measure selenium and cadmium concentrations the tissue remaining in the egg shells, with the exception of the membrane which is not easily removed, will be carefully extracted with the blunt end of a spatula. Then the contents of the Petri dish will be transferred to a blender and the egg contents homogenized. An aliquot of the homogenized sample will be analyzed for chemical concentrations. A second aliquot will be analyzed for moisture content.
- The moisture content will be standardized to a developmental time-period because eggs lose moisture during incubation.

The complete analytical protocol is included as Attachment 2.

## **DUPLICATION OF RECORDS**

At the end of each workday, all data sheets are to be given to Dan Golner. Dan will be responsible for duplication of all data forms. At the end of each week, one copy of data forms for that week will be mailed to:

**John Ratti**  
**Department of Fish and Wildlife Resources**  
**University of Idaho, Moscow, ID 83844-1136**

Copies are to be kept separate from the originals prior to mailing (e.g., originals kept in the living quarters and copies kept in the vehicle until they are mailed to Dr. Ratti).

**ATTACHMENT 1**  
**SPECIES LIST FOR BIRD EGG COLLECTION**

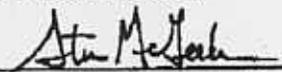
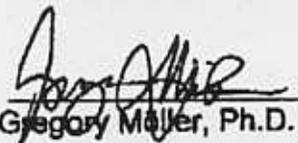
The following is the species list approved by the USFWS, IDFG, and Gray's Lake NWR for collection of eggs during the 1999 and 2000 breeding season. No more than one egg will be taken from any given nest, and no more than 40 eggs will be collected for any given species over the 2-year collection period.

- **Eared Grebe** - *Podiceps nigricollis*
- **White-faced Ibis** – *Plegadis chihi*
- **Canada Goose** – *Branta canadensis*
- **Mallard** – *Anas platyrhynchos*
- **Cinnamon Teal** – *Anas cyanoptera*
- **American Coot** – *Fuica americana*
- **Killdeer** – *Charadrius vociferus*
- **Willet** – *Catoptrophorus semipalmatus*
- **Long-billed Curlew** – *Numenius americanus*
- **Common Snipe** – *Gallinago gallinago*
- **Franklin's Gull** – *Larus pipixcan*
- **Black Tern** – *Chlidonias niger*
- **American Kestrel** – *Falco sparverius*
- **Northern Flicker** – *Colaptes auratus*
- **Horned Lark** – *Eremophila alpestris*
- **Tree Swallow** – *Tachycineta bicolor*
- **Cliff Swallow** – *Hirundo pyrrhonota*
- **Barn Swallow** – *Hirundo rustica*
- **House Wren** – *Troglodytes aedon*
- **Marsh Wren** – *Cistothorus palustris*
- **Mountain Bluebird** – *Sialia currucoides*
- **American Robin** – *Turdus migratorius*
- **European Starling** – *Sturnus vulgaris*
- **Song Sparrow** – *Melospiza melodia*
- **Red-winged Blackbird** – *Agelaius phoeniceus*
- **Yellow-headed Blackbird** – *Xanthocephalus xanthocephalus*
- **Brown-headed cowbird** – *Molothrus ater*

## **ATTACHMENT 2**

**University of Idaho  
Analytical Sciences Laboratory**

**Bird Egg Analytical Protocol**

<b>TITLE:</b> HOMOGENIZATION OF BIRD EGG CONTENTS		
<b>Contributors:</b> P. Markowski		
<b>Approved:</b>		<u>Chief Chemist</u>
	Steve McGeehan, Ph.D.	Title
		<u>7/2/99</u>
		Date
<b>Approved:</b>		<u>Technical Director</u>
	Gregory Moller, Ph.D.	Title
		<u>7-2-99</u>
		Date
<b>Filepath:</b>	P:\SOP\METHOD\INOR-GEN\EGGS.DOC	
<b>Status:</b>	<i>This document is considered current standard procedure of the Analytical Sciences Laboratory when management approval is documented by signature above. This Standard Method is effective on the date of approval signature and supersedes all other versions until historically archived by the QAU as indicated below.</i>	
<b>Archived:</b>	_____	_____
	Signature	Title
		_____
		Date
<b>Abstract:</b>		
<i>The contents of bird eggs are to be analyzed for selenium, cadmium, and percent moisture. The egg and its contents are first photographed with a digital camera and then homogenized before digestion procedures can begin. This SOP describes the procedure and equipment used.</i>		

## I. Equipment and Apparatus

### A. Equipment

1. Homogenizer/mixer, with blade attachment
2. Digital camera

### B. Miscellaneous

1. 150 x 15 mm petri dish, disposable polystyrene
2. 20 mL disposable syringes
3. Disposable 3 mL transfer pipets

## II. Sample Preparation

### A. Egg Samples

1. Crack whole eggs and carefully transfer contents, excluding the shell membrane, into a petri dish.
2. Place egg shells in the petri dish, keeping outer shell surface from contacting the egg's contents. Remove visible shell pieces from liquid contents.
3. Place the petri dish with sample on the pathology camera table. Turn on the lights that are connected to the camera table.
4. Turn off the camera flash.
5. Place an identification label, with case number and individual sample ID, directly below the petri dish.
6. To preview the picture, hold the shutter button in lightly - make sure the sample ID is visible and the shell and egg contents are all in the frame. Use the telephoto adjustment to fill screen.
7. Hold the shutter button to take the picture.

## III. Sample Homogenization

1. Egg contents that contain only yoke and other liquid contents can be homogenized with a 20 mL disposable syringe. The liquid contents must be drawn into the syringe and expelled several times to ensure the sample is homogenized to a consistent liquid form.
2. Egg contents that contain embryos are inspected for visible deformities.
3. Embryos are cut into smaller pieces with cleaned surgical scissors and with remaining liquid contents are added to a small 250 mL homogenizer/mixer container. Screw on the blade base and use the pulse mode on the homogenizer/mixer to blend egg contents.
4. Use a disposable pipet to transfer homogenized egg contents to a labelled sample container.
5. Thoroughly wash homogenizer/mixer blades and parts and finally rinse with 18 M $\Omega$ -cm water.

## IV. Equipment Blanks

1. Equipment blanks are periodically collected and submitted as a water sample to be analyzed for selenium and cadmium. After a sample has been homogenized and all the homogenizer/mixer parts washed, the homogenizer/mixer and blade assembly are rinsed with 18 M $\Omega$ -cm water and collected in a 125 mL sample bottle. A sample volume of 100 mL is of sufficient quantity for the required tests.

**ATTACHMENT 3**  
**FIELD DATA FORMS**

Selenium Project / Montgomery Watson John Ratti, University of Idaho 208-885-7741 <b>1999 Egg-Collection Data Sheet</b>			
Name		Date	
Sample Unit:	T wg	Rng	Sec
Circle One:	Stream	River	Pond/Wetland      Reservoir/Lake
Circle One:	Mining	Background	Spp Common Name
Stage of Nest: 1) Under construction   2) With eggs   3) Neonates, empty, or eggs abandoned or broken			
Number of eggs?	Eggs collected?	Yes	No
Egg ID# (Enter you initials and the sequential number of the egg collected by you.)			
Was the zip-lock collection bag labeled with the Egg ID number?		Yes	No
GPS Latitude	GPS Longitude		
Compass degrees from GPS to nest?	Distance (ft) to nest from GPS?		
GPS Location marked with flag?	Yes	No	Other?
General description of nest-location habitat: ..... ..... .....			
General description of specific nest site: ..... ..... .....			

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## Appendix B

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**PROPOSAL**

**Effects of selenium exposure on cutthroat trout (*Oncorhynchus clarki*)**

**for**

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**May 17, 1999**

## Effects of selenium on cutthroat trout

**General Objective:** To determine the effects of natural exposure to selenium through the environment on quality of cutthroat trout fry and to assess the effects of dietary intake of selenium on trout growth and reproductive performance.

- Specific Objectives:**
- (1) To determine if hatchability and percentage of malformed cutthroat trout fry differ in fish from the Blackfoot River watershed as compared to cutthroat trout from a control area.
  - (2) To evaluate the effects of dietary selenium intake on cutthroat trout growth, survival, and other performance indicators.
  - (3) To assess the effects of dietary selenium intake on reproductive performance of cutthroat trout.
  - (4) To determine if cutthroat trout from the Blackfoot River watershed lack genetic variation as compared to cutthroat populations from a control area.

### Background:

Selenium has long been known to be an essential dietary nutrient for terrestrial animals and fish (NRC, 1993). Dietary selenium deficiency causes clinical deficiency signs in rainbow trout (*Oncorhynchus mykiss*), which are limited to growth depression unless both selenium and vitamin E are deficient in the diet (Hilton et al., 1980). Similar findings are reported in channel catfish (Gatlin and Wilson, 1984). Both selenium and vitamin E are required to prevent muscular dystrophy in Atlantic salmon (Poston et al., 1976) and in rainbow trout (Bell et al., 1985). Selenium status can be determined in fish by measuring glutathione peroxidase activity, either in plasma or liver (Poston et al., 1976; Hilton et al., 1980; Bell et al., 1985, 1986, 1987). The dietary selenium requirement of rainbow trout has been estimated to be between 0.15 and 0.38 mg Se/kg diet, based upon optimum growth and maximum plasma glutathione peroxidase activity (Hilton et al., 1980). Selenium toxicity was observed in rainbow trout when dietary Se exceeded 13 mg Se/kg feed. Growth rates and feed efficiency ratios were affected by high dietary Se levels, and high mortality was observed. Nephrocalcinosis was reported in rainbow trout fed diets containing 11.4 mg Se/kg feed, with an incidence of 90% (Hicks et al., 1984). Uptake of selenium by the gills in rainbow trout is very efficient when water-borne concentrations are low (Hodson and Hilton, 1983).

The study of Hilton et al. (1980) was conducted with trout fry (1.3 g average starting weight), and continued until fish reached an average weight of 41 g. Six dietary Se treatments were used, with the highest two levels being 4.3 and 15 mg Se/kg diet (measured Se content, 3.67 and 13.06 mg/kg). No effects on growth, feed efficiency ratio

or mortality were observed in fish fed the feed containing 3.67 mg Se/kg at the end of the 20-week trial. No pathological changes were observed in fish fed the highest level of Se in the study of Hilton et al. (1980), but fish were observed to avoid feed, thus explaining the observation of reduced growth in fish fed the highest dietary level of Se. Hilton et al. (1980) suggested that prolonged ingestion of greater than 3 mg Se/kg diet may cause toxicity in rainbow trout, but provided no direct evidence to more precisely estimate the dietary Se level that would cause toxicity with prolonged dietary exposure.

More recently, a study was conducted using Atlantic salmon (*Salmo salar*) fed diets containing either 0.66 or 2.6 mg Se/kg diet (Julshamn et al., 1990). No effects on growth, feed conversion ratio, serum glutathione peroxidase activity or mortality were observed. Selenium concentrations in serum and liver were 2-4x higher in salmon fed the high-Se diet compared to those fed the low-Se diet.

The biological availability of selenium for fish differs with the selenium source. Bell and Cowey (1989) reported that the selenium present in fish meal has a low availability to rainbow trout, while that of selenomethionine is high. Lorentzen et al. (1994) observed differences in bioavailability between selenite and selenomethionine on the basis of muscle and whole-body selenium concentrations. Fish fed diets supplemented with selenomethionine had 3-5x higher muscle selenium levels than fish fed equivalent dietary selenium levels, with sodium selenite as the supplement. These observations are consistent with the explanation of Burk (1986) of the difference in uptake and metabolic fate of selenium from selenite or selenate, compared to selenium ingests as selenomethionine or selenocysteine. Selenomethionine is absorbed and metabolized as methionine; thus it can enter the tissue (muscle) pool, being incorporated into tissue protein as selenomethionine. Only when this protein is catabolized is Se released, entering the central selenium pool. High dietary methionine intake increases the proportion of dietary selenomethionine that is catabolized. Selenite, selenate, and selenocysteine all supply the central selenium pool; this is the only metabolic route available to selenium from these sources. From the selenium pool, selenium has two metabolic fates; to be incorporated into glutathione peroxidase, or into excretory metabolites. When dietary intake from selenite, selenate or selenocysteine exceeds the amount that can be excreted, homeostasis breaks down, selenium accumulates, and toxicity begins.

Research studies in fish clearly support the Burk (1986) explanation of selenium metabolism. Muscle selenium levels in Atlantic salmon are much higher in fish fed diets supplemented with selenomethionine than in fish fed diets supplemented with an equivalent amount of selenite (Lorentzen et al., 1994). Repeated observations that wild salmon smolts in the Columbia and Snake watersheds have higher whole body levels than hatchery-reared smolts is likely the result of the diet of wild salmon being composed mainly of aquatic and terrestrial insects (Higgs et al., 1995), which contain selenium as selenomethionine.

Very little information is published concerning the effects of dietary selenium on maturing fish and nothing exists in the literature concerning dietary selenium and cutthroat trout.

### **Methods and Materials:**

General Approach: Three experiments will be conducted in year one of this project. First, maturing cutthroat trout will be collected from Blackfoot River by electrofishing, and the quality of their eggs will be compared to a control group. Second, offspring from the first study will be reared for the remainder of the year in six dietary treatment groups, each receiving a feed supplemented with selenomethionine (range 1-11 mg Se/kg feed). Growth performance, glutathione peroxidase activity, and tissue Se levels will be measured in these fish. Third, genetic variation will be assessed within and among samples collected from the electrofishing effort in experiment one, and compared to other populations of cutthroat trout.

### **Experiment 1: Egg Quality**

In early June, 1999, University of Idaho (UI) personnel from the Hagerman Fish Culture Experiment Station (the Station) will undertake efforts to collect maturing cutthroat trout from Blackfoot River, led by Idaho Department of Fish and Game regional biologist, Jim Mende. Fish will be stunned by electroshocking, netted, placed in small tanks, and physically examined for spawning readiness. Females deemed to be ready to spawn, e.g. ripe, fully mature, will be gently squeezed to collect loose eggs. These eggs will be placed in labeled plastic cups, and placed in a cooler for subsequent transport to the Station for fertilization. A small piece of fin tissue will be removed from all females (and males) collected by electroshocking, whether or not they are spawned. Ripe males will be stripped to collect milt, which will be placed in labeled plastic cups, and placed in a cooler. Up to 20 females and 20 males will be spawned, with 15 fish of each gender being the minimum number that will be sampled. After examination and/or spawning, all fish will be returned to the river. The small fin clip will allow identification of these fish, should they be captured again during this collection effort.

The gametes will be returned to the Station, where they will be spawned in a controlled matrix, designed to maximize available genetic variation among offspring and minimize potential maternal or paternal effects that may potentially result from single crosses.

After fertilization and water hardening, eggs will be placed in spawning cups (ca. 500 eggs per cup), and the cups placed in a Heath tray incubator supplied with chilled (7-8°C) spring water. Within 1-2 days, the eggs will be examined for fertilization, and unfertilized eggs removed and counted. The eggs will be left undisturbed until the eyed stage (XX TU), at which time they will be shocked, dead eggs removed and counted. The eggs will then be left undisturbed until hatching (XX TU), at which time the numbers of normal, abnormal, and dead embryos (yolk-sac fry, or alevins) will be counted. Fish will

be carried forward to yolk-sac absorption, also known as the swim-up stage. At this point the number of normal, abnormal and dead fry from each cross will be counted, and the fry then pooled for early feed training and water temperature acclimation (ca. about one week). After 7-10 days of acclimation and training, the fish will be divided into 21 groups for continuation into experiment 2.

### **Experiment 2: Selenomethioine feeding trial**

**Fish and Rearing:** Cutthroat trout (average weight 100 mg) will be selected from a larger population, counted in groups of 20 fish, weighed, and placed into 21 150 l fiberglass tanks, each supplied with 1-4 l/min of untreated, constant temperature (14.5° C), spring water at the Station. Water flow to each tank will be low at first when the fish are small, but it will be increased as the fish grow. A fixed photoperiod, controlled by timer and flourescent lights, will be established at 14 hr day, 10 hr. night. The fish will be fed 10x per day, six days per week to apparent satiation, for the first several weeks of the trial. Feeding frequency will decrease as the fish grow, according to standard hatchery practice for trout. Each dietary treatment will be fed to triplicate groups of fish, positioned at random throughout the rearing system (completely randomized experimental design).

**Feeds and Feeding:** Six experimental diets will be produced at the Station first by cold extrusion, and later, as the fish grow, by compression pelleting. The formulation of the diets will be similar to commercial trout diets and the diets will contain 45% protein and 16% lipid, on an “as-is” basis. The formulation of the diets will be relatively simple, with the only difference among diets being the amount of selenomethionine added to each dietary treatment (Table 1). Samples of each feed will by taken for proximate and selenium analysis as described below. The trial will be designed as a completely randomized design for statistical evaluation of data, and, as mentioned, diets will be assigned to replicate tanks of fish in a completely randomized design. Each experimental diet will be fed to triplicate tanks of fish, but an additional treatment group will be fed the experimental diets, increasing in dietary selenium content as the fish grow. In other words, the group will be fed the unsupplemented diet (Diet 1) for the first growth period, the diet supplemented with the lowest level of selenomethionine (Diet 2) for the next growth period, and so on. Feeds will be made approximately bimonthly, and selenium levels will be analyzed to confirm that appropriate levels are present in each experimental diet before they are fed to groups of fish.

**Sampling and Analysis:** Fish in each tank will be bulk-weighed and counted every 14 days during the feeding trial from the start of the study until the fish reach an average weight of 2 g., after which they will be weighed and counted monthly. Dead fish will be removed and recorded daily. From the initial population of fish, and from two of the three replicate tank for each dietary treatment group, samples of fish will be taken at each point where the fish double in average weight, e.g. 1 g., 2 g., 4 g. and so on. The fish sample from each tank will be processed into a puree using a Robot Coupe food

processor (Robot Coupe, Moline, IL), and subsampled for proximate and selenium analysis. Proximate analysis of feed and fish samples will be conducted using standard methods: moisture by oven drying at 105°C overnight, protein by nitrogen determination using a LECO FP 428 nitrogen analyzer, crude lipid by extraction in a Goldfish apparatus with methylene chloride, and ash by incineration at 550°C in a muffle furnace. Selenium analysis will be conducted under the direction of Dr. Greg Moller at the Holm Center, University of Idaho, by ICP (Inductively-coupled argon plasma spectrophotometry). The concentration of protein, lipid, and selenium in fish at the beginning and end of each growth stanza in each dietary treatment group, and the amount of each nutrient fed during the trial, will be used to calculate apparent nutrient retention during the 84-day study.

Statistical Analysis: All appropriate data will be analyzed for statistical significance using analysis of variance (ANOVA) for the completely randomized design using GraphPad Prism, version 2.0 (GraphPad Software, Inc., San Diego, CA). A significance level of  $P < 0.05$  will be used, and tank mean values will be considered units of observation for statistical analysis. Appropriate transformations will be used on percent data (cumulative mortality) and tissue selenium concentrations if required to conform with the rules of ANOVA. If necessary, appropriate post-hoc tests will be used to identify significant differences between treatments. Regression analysis may be used to analyze growth data, if deemed appropriate.

### **Experiment 3: Genetic Analysis of Blackfoot Watershed Cutthroat Trout**

Experimental Rationale: The purpose of this experiment is to employ molecular genetic techniques to directly assess genetic variation within and among cutthroat trout in the Blackfoot Watershed. The advantage of molecular based techniques lies in the ability to quantitatively assess genotypes rather than phenotypes. Isozyme analysis has been used to examine the relatedness of individuals among and between populations of cutthroat trout. This technique as with all others, has its limitations. The level of variation and rate of mutation in isozymes may fall short of being able to detect a minimal loss of genetic variation within this population. Moreover, isozyme analyses typically require destructive sampling of tissue and organs. This aspect of isozyme analysis limits its usefulness in this situation (see Mitton 1997 for a review).

We will instead employ the use of restriction fragment length polymorphism (RFLP) analysis on a PCR amplified region of mitochondrial DNA (mtDNA). This technique is relatively simple and non-destructive. Mitochondrial DNA is inherited in a clonal (non-recombinatory) fashion from the female. This allows for direct assessment of maternal genealogy and dispersal. Mitochondrial DNA lacks similar enzymes that edit mistakes made during DNA replication in the nuclear genome. Thus, the mutation rate in mitochondrial DNA can be much higher (up to 10 fold higher) than mutation rates observed in nuclear gene sequences. These attributes, simplicity, non-destructive sampling, and high mutation rate make mtDNA RFLP analysis an attractive choice for investigating genetic variation within and among populations of cutthroat trout. Most importantly for this study, since mitochondrial DNA is only inherited from the female and

is non-recombinatory, it effectively reduces the population size to  $\frac{1}{4}$ . Thus, mitochondrial DNA is very sensitive to any potential loss of genetic variation the assesment of which is the experimental objective (see Avise 1994 for a review).

**Methods:** Fin samples from each cutthroat trout to be examined will be stored in 70% ethanol or preservation/lysis buffer until DNA is extracted using methods modified from Sambrook et al., (1989) and Dowling et al. (1990). Total genomic DNA wil be isolated from each sample and amplified using the Polymerase Chain Reaction (PCR) and nucleotide primers specific for the NADH Dehydrogenase 2 (ND2) gene region of the mitochondrial genome (ND2, #562 5'TAA GCT ATC GGG CCC ATA CC<sup>3'</sup> and #461 5'GGC TCA GGC ACC AAA TAC TAA<sup>3'</sup>). Amplification products will be digested with 15 separate restriction enzymes (*Ava* I, *Bcl* I, *Bgl* II, *Dde* II, *Dpn* II, *Hae* III, *Hha* I, *Hinc* II, *Hind* III, *Hinf* I, *Mse* I, *Msp* I, *Nhe* I, *Pvu* II, and *Rsa* I). The resulting mtDNA fragments are separated by electrophoresis using 3% agarose/TAE gels. Vertical 6% polyacrylamide/TBE gels are also used to separate small fragments and questionable co-migrating fragments. Photographs of each gel are converted into computer image files using a ScanMan scanner and ScanMan 2.0 software (Logitech). Restriction fragment length polymorphisms (RFLPs) observed among samples are visualized using SigmaScan Pro 3.0 (Jandel Scientific 1996), then given alphabetical designations as haplotypes. The size of each DNA fragment from each mtDNA gene region is estimated by comparison to a size standard, pUC-19 marker (Bio-Synthesis). Alphabetical designations for RFLPs of each mtDNA gene region are combined into composite mtDNA haplotypes. The resulting composite haplotypes will be compared to composite haplotypes from populations of two other populations of cutthroat trout (*Oncorhynchus clarki bowvieri*) and a different subspecies, westslope cutthroat trout (*Oncorhynchus clarki lewsi*) to add geographic and phylogenetic perspective to the analysis.

**Population and Statistical Analyses:** An estimate of the number of nucleotide substitutions per site ( $p$ ) for each RFLP is calculated via the Nei (1987) method using REAP 4.0 (Restriction Enzyme Analysis Package) (McElroy et al. 1991) and then used to generate a matrix comparing  $p$  values (distance) between all pairs of identified composite haplotypes. The KITSCH program in PHYLIP 3.5 (Felsenstein 1993) which assumes independence and equal rates of divergence is used to generate an distance dendrogram using the least-squares method of Fitch and Margoliash (1967) to illustrate the estimated evolutionary relationships and distance among the identified composite haplotypes. The extent of geographic heterogeniety among population frequency distributions will examined using a Monte Carlo simulation of a chi-square analysis with 1000 iterations (MONTE program in REAP ver. 4.0). Nucleotide diversity and divergence among and within populations will be estimated using Nei (1987) equations 10.19, 10.7, 10.20, and 10.21 in the DA program of REAP ver. 4.0. The resulting matrix of nucleotide divergence among populations is used to construct a neighbor-joining tree using NTSYS-pc 1.80 (Roff 1993).

Thus, the genetic relationship of Blackfoot River cutthroat trout to two nearby populations will be ascertained along with the extent of genetic variation as it relates to mitochondrial DNA variation. The level of genetic variation in the Blackfoot population

will be compared to that of the other populations to assess whether or not a loss of variation has occurred.

**Time Table:** A 12-month period is required to obtain eggs and tissue samples, conduct the feeding trial, and complete the genetic analysis. With respect to the various experiments, experiment 1 will commence on June 1, 1999, and be completed by the end of July, 1999. Experiment 2 will commence in the second half of July, 1999, when the cutthroat trout will likely be ready to begin in the feeding trial, and continue throughout the funding period. Experiment 3 will begin in June, 1999, when sampling begins, and be completed by February 29, 2000.

**Products of Research:** Progress reports will be produced at the end of each quarter, e.g. September 1, 1999, December 1, 1999, March 1, 2000 of the budget year (June 1, 1999) and will contain data sheets and tables of growth and performance results. A final report will be produced by April 15, 2000, and contain all fish performance and chemical analysis results through February, and conclusions and recommendations for further research.

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**Proposed Annual Work Schedule:** June 1, 1999 to May 31, 2000.

June	Obtain fish, spawn, and incubate Take fin clips for genetic analysis
June	Prepare experimental feeds
July	Fry hatch
July-August	Begin feeding trial
August-September	First sampling (fish ca. 1 g)
September	Second sampling (fish ca. 2 g)
October	Third sampling (fish ca. 4 g)
November	Fourth sampling (fish ca. 8 g)
December	Fifth sampling (fish ca. 16 g)
January	Sixth sampling (fish ca. 32 g)
February	Seventh sampling (fish ca. 64 g)
March-April	Eighth sampling (fish ca. 128 g)
May-June	Ninth sampling (fish ca. 256 g)

Table 1. Proposed composition\* of experimental diets 1-6 (g/kg diet).

<u>Ingredient</u>	<u>Diets 1-6</u>
Fish meal (LT Icelandic capelin)	600
Wheat middlings	254
Fish oil	123
Ascorbic acid (phosphate ester)	1
Choline Chloride (70% liquid)	6
Trace mineral premix	1
Vitamin premix	15

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\* all diets mixed as one big batch, divided into six equal batches, and selenomethionine (Sigma, St. Louis) added as follows per kg diet (dry basis): Diet 1, none; Diet 2, 2 mg Se; Diet 3, 4 mg; Diet 4, 6 mg; Diet 5, 8 mg; Diet 6, 10 mg. We estimate that the basal diet will contain between 1-2 mg Se/kg.

Calculated proximate composition (% as-is basis)

Moisture	6.30
Crude protein	45.0
Crude fat	16.0
Ash	10.3

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## Appendix C

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## **DEAR CONTROLLED HUNT PARTICIPANT:**

The Interagency/Industry Selenium Working Group is collecting information on the mineral status of big game in Management Unit 76. We are particularly interested in selenium levels in free-ranging elk. Selenium is a naturally occurring, nutritionally required trace mineral. Through soil, water, and vegetation sampling over the last two years, we know that elevated selenium levels exist in some locations associated with phosphate mining. We are interested in testing animals from various locations in Unit 76 to compare with soil, water, and vegetation sampling.

## **YOU CAN HELP!!**

Liver samples provide one method of measuring mineral levels in big game and other animals. We are asking each successful hunter to voluntarily collect a baseball-sized piece of liver from the harvested animal and place it in the small plastic bag provided. After collecting, please try to keep the sample on ice or as cool as possible. Freezing the sample is okay.

## **DROP OFF LOCATIONS**

Idaho Department of Fish and Game is assisting the Selenium Working Group in the collection of livers and will have check stations set up at the following locations and times. In addition to collecting liver samples, personnel at the check stations will also collect a small meat sample from your animal and gather information on the location of the kill.

<u>Locations</u>	<u>Dates</u>	<u>Times</u>
1.) U.S. 34 just north of Soda Springs	Oct. 26,27,30,31 and Nov. 6,7	10am. to 6pm.
2.) Lower Georgetown Canyon Road	Oct. 26,27,30,31 and Nov. 6,7	10am. to 6pm.

If you would like to drop off a liver sample but are unable to make it to one of the check stations, you can bring it by the Idaho Department of Fish and Game office in Pocatello (1345 Barton Road) between 8am. and 5pm, M-F. You can also drop it by the U.S. Forest Service office in Soda Springs (421 West 2<sup>nd</sup> South) or Montpelier (322 N. 4<sup>th</sup> St.) from 8am to 4:30 pm, M-F. If you have any questions, please call Idaho Fish and Game at 208-232-4703.

## **THANK YOU IN ADVANCE !!**

The Cooperators of the Interagency/Industry Selenium Working Group

Solutia, Inc.	Idaho DEQ	Idaho Dept. of Lands
Nu-West Industries, Inc.	Idaho Fish and Game	U.S. Fish and Wildlife Service
J.R. Simplot Company	U.S. Forest Service	Shoshone-Bannock Tribes
FMC Corporation	U.S. Bureau of Land Management	
Rhodia, Inc.		





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## Appendix D

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# Research Proposal and Protocol

October 6, 1999

Title: Evaluation of selenium levels in blood, skeletal muscle and liver tissue from steers confined to grazing reclaimed phosphoria mine sites in southeastern Idaho.

Desired Starting Date: October 1, 1999 (arrival of steers to University of Idaho facilities)

Duration of Project: 120 days (approximate duration of steers at UI)

## Introduction

### Background Information:

During the months of June-August of 1999, 45 yearling steers were restricted to graze on outside waste dumps that were reclaimed nearly 20 years ago. The study site is located at the South Henry Mine, near Soda Springs in southeastern Idaho. The soil and vegetation are known to have elevated selenium concentrations. The steers were allowed to graze this area for a period of 9 weeks during the summer. Blood from the steers and vegetation and soil samples were taken during the 9-week grazing study. Blood samples were collected at two-week intervals from 15 randomly selected steers and analyzed for whole blood and serum selenium levels. Vegetation from the study site was clipped at the two-week blood collection intervals and analyzed for selenium. Soil samples were collected at the beginning and end of the grazing period and analyzed for selenium. After the 9-week period, the steers were removed from the reclaimed outside waste dump and were allowed to graze on off-site native pasture for approximately 30 days. Off-site blood draws were continued for the month of September at approximately two-week intervals. Off site native vegetation was sampled for selenium. On October 1, 1999 the 15 study steers and 5 control steers will be brought to the University of Idaho Beef Center, Moscow, Idaho, for selenium tissue studies and continuation of the selenium blood study.

### Problem Statement:

Elevated levels of selenium can potentially pose human health concern when allowed to enter the food chain unchecked. A depuration rate of elevated levels of selenium in blood and tissue from livestock is not completely understood.

### Objectives:

The objectives of this proposal will be four fold:

1. Continuation of the summer grazing study to sample for whole blood and serum selenium levels every two weeks.
2. Determine selenium levels at two intervals in liver tissue and skeletal muscle during a simulated feedlot situation.
3. Determine depuration rates of selenium from blood, liver, and muscle tissue during a 120-day feedlot simulation trial.
4. Sample liver, kidney, and heart tissue and collect a representative sample of all major muscle groups at the time of slaughter.

### Justification:

Selenium cycling from reclaimed mine vegetation to livestock tissue concentration is poorly understood. Due to the sensitivity and concern regarding livestock selenium levels and their role in the food chain, further investigations need to be generated from ongoing research projects.

### Methods

At the UI Beef Center the steers will be allowed to acclimate for 7 days before any surgical procedure or feedlot ration begins. Steers will be fed a finish ration that will closely mimic commercial feedlot situations. The finish ration will be analyzed for basal selenium concentrations. The steers will be bled every two weeks for the duration of the study. Serum and plasma taken from the blood samples will be analyzed for selenium. Dr. Steve Parish, Washington State University veterinarian will conduct muscle and liver biopsy at days 7, 50, and 100. At day 120-post arrival to the UI Beef Center, all 20 steers will be slaughtered at the UI Meats Laboratory. Further sampling of muscle and tissue will be taken at the time of slaughter. Dr. Parish will submit blood and tissue samples to the UI Analytical Sciences Laboratory.

### Muscle Biopsy:

The surgical procedure, muscle biopsy, is considered a minor surgical event; therefore no specific preoperative considerations are generally made other than the animals normal well being. Since the procedure is a minor one, no pre-op antibiotics will be given. The animals are considered well vaccinated. The biopsies will be performed under local infiltrative lidocaine anesthesia. Approximately 4-5 ml. of 2 % lidocaine will be infiltrated subcutaneously to provide local anesthesia for a stab incision and muscle biopsy.

Tissue will be taken from the biceps femoris muscle. The amount of 1-2.25 grams will be sufficient. After a 10 cm X 10 cm area is clipped over the biceps femoris, a standard surgical preparation of the site will be done. This will be followed by lidocaine infiltration. Once analgesia is established, a 1-cm incision will be made longitudinally through the skin. After the incision is complete, the muscle biopsy instrument will be inserted and the biopsy obtained. One to two skin sutures will be placed in the skin to close the incision. Sutures will be removed in 2 weeks. The site will be observed daily for abnormal swelling, dehiscence or other abnormalities.

Although the surgery is minor, all procedures will be done aseptically using sterile instruments, needles, sutures and gloves. Instruments will be chemically sterilized between animals using an appropriate Nolvosan solution. The animals will be observed by a qualified individual daily to determine if incisions are healing appropriately. If a

problem occurs, the infected area will be assessed and appropriate antibiotics, drainage, etc. will be provided.

#### Liver Biopsy:

Steers will be given a thorough physical examination, especially to evaluate any hemostatic defects. The cattle will be adequately restrained in a squeeze chute with a headgate and nose thong. The skin over the biopsy site should be clipped and prepared for aseptic insertion of the needle. The site is anesthetized using a local infiltration of 2 % lidocaine of 2-4 mls. A small stab incision is made through the skin at the site of insertion with a #10 Bard Parker blade. The puncture site can be located by extending a horizontal line cranial from the middle of the paralumbar fossa. The needle is inserted where this line crosses the 11th intercostal space on the right side. A disposable "Tru Cut" needle is passed through the skin incision and directed slightly cranial and ventrad. Care is used to insert the needle near the anterior aspect of the 12th rib so as to avoid the intercostal artery as it courses along the posterior aspect of the 11th rib. Successful penetration of the liver will be felt as a slight resistance. The steers will be observed post biopsy for any evidence of bleeding or shock.

#### Timetable

Twenty steers will arrive at the UI Beef Center on October 1, 1999. To adequately simulate a feedlot situation, the animals will be fed a finishing ration for nearly 120 days.

#### Personnel

The project will be an extension to a master's thesis for Jeff Knight. Dr. James Kingery, University of Idaho, Rangeland Ecology and Management is the project coordinator. Dr. Steve Parish from Washington State University will conduct all liver and tissue biopsies as well as general animal health evaluations. Dr. Carl Hunt, University of Idaho, will assist in feed ration formulation and act as a liaison with personnel at the beef center. Dr. Patricia Talcott and Dr. Greg Moller will oversee tissue analysis procedures and protocols at the UI Analytical Sciences Laboratory. Other cooperators are Denny Falk, UI Beef Center Manager; Ron Lewis, Feed Mill Manager and Ron Richards, UI Meats Laboratory Manager.

#### Facilities

The facilities at the University of Idaho are well suited in all phases of the proposed research. The Agriculture Department has modern holding facilities with excellent working corrals and squeeze chute. The facilities are equipped for research purposes and therefore provide tailored needs to successfully complete the project. Located at the UI Beef Center is a feed mill for tailored ration formulation proposed. Also located at the beef center is a modern USDA federally inspected abattoir used for teaching and research purposes. The UI Analytical Sciences Laboratory (ASL) is a full-service laboratory, which operates in the College of Agriculture at the University of

Idaho. Scientists and support staff at the ASL are highly qualified to conduct proper analytical methods.

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## Appendix E

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**APPENDIX E**  
**Raw Laboratory Data for the 1999 Interim Investigation**

The accompanying diskette contains an Excel 95 file with 4 worksheets, one each for the May 1999 water column, monthly water column (June-August, 1999), elk tissue, and beef post-mortem tissue data. Each worksheet represents Montgomery Watson's working copy of the raw laboratory data. With the exception of the beef data, which were provided to Montgomery Watson in hard copy form only, each worksheet represents the digital output of the University of Idaho's laboratory information management system (LIMS). The LIMS output as received has been modified as follows:

- Below detection limit (BDL) values have been replaced with instrument read-outs. If the value in the results column is less than the value in the equipment detection limit (EDL) column, the laboratory reported the result as BDL;
- Non-detect (ND) values have been replaced with zeroes;
- Blank data for May water column sampling (cadmium and major ions) were inadvertently omitted, thus calibration blank data have been added at the end of the May water worksheet;
- Matrix spike and blank spike results reported as percentages have been replaced with the actual concentrations observed; and,
- A column of known laboratory standard and matrix spike concentrations has been added.

A hard copy of the LIMS output, the corresponding quality control (QC) records, and instrument read-outs, is maintained in the project file at Montgomery Watson office in Bellevue, Washington, and a duplicate copy has been provided to Idaho Department of Environmental Quality's Pocatello, Idaho office.

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## Appendix F

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**TABLE F-1**  
**1999 INTERIM INVESTIGATION VALIDATED STREAM WATER COLUMN DATA**

Station ID	Date	Sample ID	UI Sample	Selenium	Cadmium	Iron	Calcium	Magnesium	Sodium	Potassium	Alkalinity as CaCO <sub>3</sub>	Chloride	Sulfate
				(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
<b>May 1999 Stream Sampling Results</b>													
ST019	5/25/99	052599SWST019-0-U	E9900099	<b>0.0082</b>	<i>-0.00073</i>	<i>-0.019</i>	<b>53</b>	<b>8.8</b>	<b>3.6</b>	<i>1.1</i>	<i>150</i>	<b>1.9</b>	<b>12</b>
ST020	5/25/99	052599SWST020-0-U	E9900101	<b>0.0072</b>	<i>-0.00073</i>	<b>0.070</b>	<b>53</b>	<b>9.8</b>	<b>3.6</b>	<i>1.3</i>	<i>150</i>	<b>1.9</b>	<b>11</b>
ST022	5/25/99	052599SWST022-0-U	E9900100	<b>0.0098</b>	<i>0.0000</i>	<i>-0.021</i>	<b>52</b>	<b>9.6</b>	<b>3.2</b>	<i>0.69</i>	<i>140</i>	<b>1.6</b>	<b>11</b>
ST023	5/24/99	052499SWST023-0-U	E9900111	<b>0.0078</b>	<i>-0.0015</i>	<b>0.18</b>	<b>50</b>	<b>9.2</b>	<b>2.9</b>	<i>1.5</i>	<i>130</i>	<b>1.4</b>	<b>9.8</b>
ST024	5/24/99	052499SWST024-0-U	E9900105	<b>0.0074</b>	<i>0.00073</i>	<b>0.24</b>	<b>52</b>	<b>9.5</b>	<b>3.1</b>	<i>1.5</i>	<i>120</i>	<b>1.1</b>	<b>7.7</b>
ST026	5/25/99	052599SWST026-1-U	E9900094	<b>0.0080</b>	<i>-0.0012</i>	<i>-0.018</i>	<b>49</b>	<b>8.0</b>	<b>3.0</b>	<i>0.83</i>	<i>140</i>	<b>1.3</b>	<b>8.9</b>
ST029	5/26/99	052699SWST029-0-U	E9900102	<i>0.00048</i>	<i>0.00073</i>	<i>0.0076</i>	<b>48</b>	<b>8.2</b>	<b>3.2</b>	<i>1.4</i>	<i>140</i>	<b>1.2</b>	<b>7.2</b>
ST113	5/24/99	052499SWST113-1-U	E9900106	<b>0.044</b>	<i>0.0011</i>	<b>0.066</b>	<b>75</b>	<b>13</b>	<b>5.3</b>	<i>1.7</i>	<i>160</i>	<b>7.0</b>	<b>71</b>
ST145	5/25/99	052599SWST145-0-U	E9900113	<b>0.046</b>	<i>-0.0015</i>	<b>0.19</b>	<b>54</b>	<b>10</b>	<b>2.9</b>	<i>1.2</i>	<i>160</i>	<b>1.5</b>	<b>12</b>
ST229	5/25/99	052599SWST229-0-U	E9900112	<b>0.019</b>	<i>-0.00073</i>	<b>0.18</b>	<b>51</b>	<b>9.5</b>	<b>2.9</b>	<i>1.4</i>	<i>150</i>	<b>1.2</b>	<b>9.5</b>
ST232	5/24/99	052499SWST232-0-U	E9900104	<b>0.0067</b>	<i>-0.00073</i>	<b>0.33</b>	<b>58</b>	<b>11</b>	<b>3.8</b>	<i>1.5</i>	<i>170</i>	<b>2.2</b>	<b>13</b>
ST233	5/26/99	052699SWST233-0-U	E9900103	<i>0.00049</i>	<i>0.0015</i>	<i>-0.047</i>	<b>59</b>	<b>21</b>	<b>10</b>	<i>2.5</i>	<b>200</b>	<b>9.8</b>	<b>25</b>
<b>June 1999 Stream Sampling Results</b>													
ST113	6/23/99	062399SWST113-0-U	E9900384	<i>0.0068</i>	<i>0.000784</i>	<i>0.13</i>	<i>82</i>	<i>16</i>	<i>6.2</i>	<i>1.5</i>	<i>190</i>	<i>5.8</i>	<i>61</i>
ST232	6/23/99	062399SWST232-1-U	E9900385	<i>0.0021</i>	<i>0.00010</i>	<i>0.015</i>	<i>65</i>	<i>15</i>	<i>4.3</i>	<i>1.2</i>	<i>200</i>	<i>2.0</i>	<i>11</i>
<b>July 1999 Stream Sampling Results</b>													
ST113	7/21/99	072199SWST113-0-U	E9900506	<i>0.0027</i>	<i>0.0012</i>	<i>-0.020</i>	<i>84</i>	<i>16</i>	<i>6.7</i>	<i>3.4</i>	<i>210</i>	<i>10</i>	<i>7.5</i>
ST232	7/21/99	072199SWST232-1-U	E9900507	<i>0.0024</i>	<i>0.0016</i>	<i>-0.030</i>	<i>60</i>	<i>16</i>	<i>4.5</i>	<i>1.3</i>	<i>200</i>	<i>4.3</i>	<i>3.2</i>
<b>August 1999 Stream Sampling Results</b>													
ST113	8/10/99	081099SWST113-1-U	E9900553	<i>0.0012</i>	<i>0.0018</i>	<i>-0.0075</i>	<i>85</i>	<i>18</i>	<i>6.7</i>	<i>1.6</i>	<i>210</i>	<i>4.3</i>	<i>74</i>
ST232	8/10/99	081099SWST232-0-U	E9900559	<i>0.0016</i>	<i>0.00088</i>	<i>0.029</i>	<i>58</i>	<i>16</i>	<i>4.7</i>	<i>1.4</i>	<i>200</i>	<i>2.0</i>	<i>12</i>

Notes: Data corrected, in the sequence presented here, for lab blanks, lab-standards slope, field blanks, and matrix-spike slope; mean reported for stations with replicate samples.  
 Bolded values exceed the 95% upper confidence limit of the 95th percentile of blank results and therefore are discernibly different from a blank.  
 Italicized values do not exceed the 95% upper confidence limit of the 95th percentile of blank results and therefore are not discernibly different from a blank.  
 Values that are neither bolded nor italicized do not have a corresponding 95% upper confidence limit of the 95th percentile of blank results due to a small number of blanks.

**TABLE F-2**  
**INTERIM 1999 INVESTIGATION**  
**STREAM HARDNESS DATA RESULTS**  
**FOR CADMIUM**

Site ID	Date	Sample ID	UI Sample	Hardness (mg CaCO <sub>3</sub> /l)	Harness-Specific Criterion for Cadmium (mg/l)
<b>May 1999 Stream Hardness Results</b>					
ST019	5/25/99	052599SWST019-0-U	E9900099	170	0.0015
ST020	5/25/99	052599SWST020-0-U	E9900101	170	0.0015
ST022	5/25/99	052599SWST022-0-U	E9900100	170	0.0015
ST023	5/24/99	052499SWST023-0-U	E9900111	160	0.0015
ST024	5/24/99	052499SWST024-0-U	E9900105	170	0.0015
ST026	5/25/99	052599SWST026-1-U	E9900094	160	0.0015
ST029	5/26/99	052699SWST029-0-U	E9900102	150	0.0014
ST113	5/24/99	052499SWST113-1-U	E9900106	240	0.002
ST145	5/25/99	052599SWST145-0-U	E9900113	180	0.0016
ST229	5/25/99	052599SWST229-0-U	E9900112	170	0.0015
ST232	5/24/99	052499SWST232-0-U	E9900104	190	0.0017
ST233	5/26/99	052699SWST233-0-U	E9900103	230	0.0019
<b>June 1999 Stream Hardness Results</b>					
ST113	6/23/99	062399SWST113-0-U	E9900384	270	0.0021
ST232	6/23/99	062399SWST232-1-U	E9900385	220	0.0018
<b>July 1999 Stream Hardness Results</b>					
ST113	7/21/99	072199SWST113-0-U	E9900506	280	0.0022
ST232	7/21/99	072199SWST232-1-U	E9900507	220	0.0018
<b>August 1999 Stream Hardness Results</b>					
ST113	8/10/99	081099SWST113-1-U	E9900553	290	0.0023
ST232	8/10/99	081099SWST232-0-U	E9900559	210	0.0018

**TABLE F-3**  
**INTERIM 1999 INVESTIGATION STREAM FIELD DATA**

Site ID	Date	Sample ID	UI Sample	pH (units)	Conductivity (uS/cm)	Temperature (°C)	Dissolved Oxygen (mg/l)	Turbidity (NTU)	Redox (mV)
<b>May 1999 Stream Field Data</b>									
ST023	5/24/99	052499SWST023-0-U	E9900111	8.2	300	13.1	9.3	103	165
ST024	5/24/99	052499SWST024-0-U	E9900105	8.2	297	12.4	8	98	148
ST113	5/24/99	052499SWST113-1-U	E9900106	8.1	447	17.23	7.8	10.3	118
ST232	5/24/99	052499SWST232-0-U	E9900104	8	340	14.6	7.7	62	112
ST019	5/25/99	052599SWST019-0-U	E9900099	8.2	262	16.2	8.1	66.3	97
ST020	5/25/99	052599SWST020-0-U	E9900101	8.1	310	15.1	8	88.3	206
ST022	5/25/99	052599SWST022-0-U	E9900100	8.2	234	13.3	8.6	113	165
ST026	5/25/99	052599SWST026-1-U	E9900094	8.2	292.3	11.6	8.9	106	108
ST145	5/25/99	052599SWST145-0-U	E9900113	8	317	11	9.1	110	131
ST229	5/25/99	052599SWST229-0-U	E9900112	8.2	299	9.8	9	109	131
ST029	5/26/99	052699SWST029-0-U	E9900102	nm	282	11.1	8.8	104	178
ST233	5/26/99	052699SWST233-0-U	E9900103	8.4	435	14.8	9.1	14.4	198
<b>June 1999 Stream Field Data</b>									
ST113	6/23/99	062399SWST232-0-U	E9900384	7.8	379.7	13.6	8.06	3.93	nm
ST232	6/23/99	062399SWST232-1-U	E9900385	8.3	326.5	15.9	8.51	7.62	200
<b>July 1999 Stream Field Data</b>									
ST113	7/21/99	072199SWST113-0-U	E9900506	8.02	506	20.5	7.79	12.4	nm
ST232	7/21/99	072199SWST232-1-U	E9900507	8.37	362.67	22.1	8.83	13.53	nm
<b>August 1999 Stream Field Data</b>									
ST113	8/10/99	0810/99SWST113-1-U	E9900553	8.34	534.33	17.93	10.94	13.2	nm
ST232	8/10/99	081099SWST232-0-U	E9900559	8.25	391	21.2	8.61	11.1	
Notes: nm - not measured									

**Appendix F**  
**May 1999 Surface Water Data Validation Parameters**

Analyte	m <sub>L</sub>	b <sub>L</sub>	m <sub>F</sub>	b <sub>F</sub>
Se	1.010	0.00002000	1.124	-0.0001283
Cd	0.9935	-0.01125	1.374	0.001036
Ca	1.004	0.01250	1.000*	0.1370
Mg	0.9707	-0.02000	1.000*	0.003606
K	0.9553	0.7000	1.000*	-0.2674
Na	0.9760	0	1.000*	-0.01691
Fe	1.060	-0.02000	1.000*	0.01344
Alk	1.069	0	1.000*	5.708
SO <sub>4</sub>	1.002	0.07033	0.8985	0.04459
Cl	1.020	0.1617	1.079	-0.1267

\*No matrix spikes; therefore, a default value of 1.000 is used.

### Appendix F

#### CALCULATION OF UTB VALUES FOR MAY 1999 STREAM DATA

Analyte	Lab Blanks <sup>1</sup>				Equipment Blanks <sup>2</sup>				F-Test (1-sided; $\alpha = 0.05$ )				t-Test (2-sided; $\alpha = 0.05$ )			UTB ( $\alpha = 0.05$ ; $p = 0.95$ , $n = v_{pooled} + 1$ )		Notes
	n	v	$\bar{x}$	s	n	v	$\bar{x}$	s	F	$p^3$	$S_{pooled}$	$V_{pooled}$	t	p	$\bar{x}_{pooled}$	g'	UTB	
Se	2	1	0	0.0003221	2	1	0	0.00007538	0.05	0.85	0.0002339	2	0.000	1.00	0	7.656	0.0018	
Cd	4	3	0	0.002378	2	1	0	0.001036	0.19	0.69	0.002124	4	0.000	1.00	0	4.203	0.0089	
Ca	4	3	0	0.009574	2	1	0	0.05635	34.65	0.01						26.260	1.5	Only equipment blanks used <sup>4</sup>
Fe	4	3	0	0.008165	2	1	0	0.01100	1.82	0.27	0.0090	4	0.000	1.00	0	4.203	0.038	
K	4	3	0	0.5706	2	1	0	0.7483	1.72	0.28	0.6198	4	0.000	1.00	0	4.203	2.605	
Mg	4	3	0	0	2	1	0	0.002185	#DIV/0!	#DIV/0!	0.0011	4	0.000	1.00	0	26.260	0.0574	
Na	4	3	0	0.06733	2	1	0	0.06303	0.88	0.42	0.0663	4	0.000	1.00	0	4.203	0.2786	
Alk	1	0	0	0	2	1	0	6.484	#DIV/0!	#DIV/0!	6.4842	1	0.000	1.00	0	26.26	170	
Cl	3	2	0	0.1863	2	1	0	0.06866	0.14	0.75	0.1572	3	0.000	1.00	0	5.144	0.8088	
SO <sub>4</sub>	3	2	0	0.09623	2	1	0	0.1624	2.85	0.23	0.1223	3	0.000	1.00	0	5.144	0.6292	

Notes: 1) Laboratory-corrected results.

2) Field-and-laboratory corrected results.

3) When the p-value is greater than 0.05 the means can not be pooled. Equipment blank standard deviation used because it represents greater error estimate.

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## **Appendix G**

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**Avian Study Reproductive Success Task for  
Year 2000 Field Work**

**Idaho Mining Association Selenium Committee  
Southeast Idaho Phosphate Resource Area  
Selenium Project**

Proposal submitted to:

**William Wright, Project Manager  
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Proposal submitted by:

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March 2000

## 1.0 INTRODUCTION

The 1999 egg data suggest that levels of selenium in some avian eggs collected on mining sites might be elevated when compared to eggs from the background area. Consequently, a new task will be added to the avian egg study for the 2000 field season to consider reproduction success, i.e., are the elevated levels of selenium causing a reproductive performance? To attempt to answer this question, key reproductive parameters including clutch size, hatching success, fledging success, and post-fledging survival will be examined. Other measures of reproductive success might include weight of eggs at a specific stage of incubation, and weight of neonates at a specific pre-fledging age. Details of the reproductive success task are presented below.

### 1.1 ANALYSIS OF REPRODUCTIVE PARAMETERS OF SELECTED AVIAN SPECIES

Reproductive depression is a significant effect of selenium toxicity in birds. Specific effects noted in the literature are embryonic deformities (i.e., teratogenesis) and decreased hatching success (Skorupa 1998). A decrease in post-hatch survivorship can also be suspected.

To investigate potential effects of selenium toxicity on birds in southeast Idaho, nests for five (5) or six (6) species in both the mining and control areas will be monitored. Potential target species include:

- American coot;
- American robin
- barn swallow;
- red-winged blackbird; and,
- yellow-headed blackbird.

An excess of 20 nests for each species from both the mining and control areas will be monitored. These species were selected because they are common on the study area (Ratti 2000), their nests are easily found and monitored (Rocklage et al. 2000), they rarely desert nests due to human interference (Ortega et al. 1997), and they are rarely parasitized by brown-headed cowbirds (Ehrlich et al. 1988). Red-winged blackbirds can be commonly parasitized; however, it is believed that field crews will be able to obtain a large sample of nests that are not parasitized or that the parasitism effect can be partitioned out. Mallards and song sparrows were not selected because they may easily abandon nests due to human disturbance. Song sparrows are also commonly parasitized by cowbirds; furthermore, the eggs of song sparrows and cowbirds are very similar (Baicich and Harrison 1997) and some field researchers may not be able to differentiate them, which could bias results.

Reproductive parameters that will be examined and compared between mining and control areas include:

- clutch size;
- egg weights;
- hatching success (number of eggs that hatch/clutch)
- chick weights;
- fledging success (number of chicks that fledge/number of chicks that hatch);
- survival rates of eggs and chicks (Mayfield estimators); and,
- post-fledging survival.

It will not be possible to examine all of these parameters for each species. For example, field researchers may not be able to monitor coot chicks after hatching or songbirds after fledging without marking or color-

banding chicks. As a minimum for songbirds, researchers will examine clutch size, hatching success, fledging success, and survival rates of eggs and chicks. For coots, clutch size, hatching success, and survival rates of eggs will be examined. It is anticipated that researchers will be able to weigh eggs and chicks for only the red-winged blackbirds. Since selenium may effect the physiology and behavior of the female, researchers will also compare rates of abandonment and desertion.

Nest searching and monitoring will follow recommendations of Martin and Geupel (1993) and Ralph et al. (1993). Each area will be searched for nests once every three (3) to four (4) days. When a nest is found, a detailed description of the nest location will be recorded, including nest height and species of plant in which the nest is placed. A small piece of surveyor tape will be placed  $\geq 20$  m from the nest to facilitate nest location for subsequent nest checks. Nests will be monitored with as little disturbance as possible, particularly during nest building and egg-laying when females are most likely to abandon nests (Martin and Geupel 1993). Nests will be checked every three to four days to determine nest status (e.g., incubation, chicks in nest, depredated). Nests near fledging will be monitored every one (1) to two (2) days to accurately determine fledging success. Researchers will carefully count eggs and/or chicks (including those of brown-headed cowbirds) each time the nest is checked. The researchers will also examine chicks for evidence of teratogenesis, and any deformities will be photographed. Nests above eye level will be checked with a mirror on an extension pole.

Researchers will attempt to follow coot broods post-hatching and American robins post-fledging. Again, this may be difficult without marked individuals. However, young of both species are known to remain in the natal area and aggressive parental defense will assist in relocating young. Furthermore, neither are colonial nesters (such as red-winged and yellow-headed blackbirds); therefore, it should be possible to track individual family groups. Researchers will attempt to relocate young once every three to four days for at least two weeks after hatching/fledging.

Eggs of red-winged blackbirds will be weighed near hatching (day nine (9) of the 10-12 day incubation period). Each egg will be individually marked with a non-toxic marker. Nests will be monitored daily near hatching to correlate egg weight with hatching success. To avoid chicks prematurely leaving the nest due to disturbance, chicks will be weighed at day eight (8) of the 11-14 day nestling period. Eggs and chicks will be handled with surgical gloves and will be weighed in padded containers.

One of nine fates (abandoned, deserted, depredated, lost to weather, fledged only brown-head cowbird young, fledged host and cowbird young, fledged only host young, and unknown) will be assigned to each nest. Ehrlich et al. (1988) and Baicich and Harrison (1997) will be used for references for lengths of incubation and nesting cycles. Abandoned nests are those that are found during building, but, in which eggs are not laid. Deserted nests are those without the presence of parents and in which eggs remain in the nest longer than the average incubation period for that species. A nest will be considered depredated if the eggs or chicks disappear before the nest is due to fledge. Successful nests are those that fledge at least 1 host chick. Fledged nests are those in which researchers either observe the actual fledging event, fledglings are observed near the nest, or the parents are defensive and/or carrying food near the empty nest. In addition, a nest will be considered fledged if the mid-point date between the last day the nest had chicks and the final check when the nest was empty is within two days of predicted fledge date, but only if there are no clues the nest was depredated.

The Mayfield method (Mayfield 1975, Johnson 1979, Hensler and Nichols 1981) will be used to calculate daily-survival rates and nest success for each species on mining and control areas. Failed nests are those that are lost to desertion, weather, cowbirds, or predation. Abandoned nests are not used in Mayfield calculations because eggs were not laid (Mayfield 1975); however, rate of abandonment will be compared between mining and control area. Nesting-cycle lengths will be obtained from Ehrlich et al. (1988). Daily-survival rates between mining and control areas will be compared with a  $X^2$  analysis in the program CONTRAST (Sauer

and Williams 1989). Daily-survival rates will also be investigated to determine whether they differ between the incubation and nestling period (from hatching to fledging).

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## Appendix H

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**APPENDIX H**  
**1999 ELK TISSUE LABORATORY ANALYSIS RESULTS**

IDFG License Number	Sample Type		Map ID	General Kill Location	Kill Date	Sex	Age		Liver Moisture Content (%)	Muscle Moisture Content (%)	Selenium				Cadmium			
											Liver		Skeletal Muscle		Liver		Skeletal Muscle	
	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)					Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)			Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)				
	< 1 Year	> 1 Year																
101-9-00113	X	X	1	Diamond Flat	10/26/99	M		X										
101-9-001192	X	X	2	Sage Creek	10/28/99	F		X	69	73	<b>0.53</b>	1.7	<b>0.12</b>	0.44	<b>0.39</b>	1.3	<b>0.084</b>	0.31
101-9-003717	X	X	3	Buck Mountain	11/6/99	F		X	67	70	<b>13</b>	38	<b>0.36</b>	1.2	<b>0.26</b>	0.79	<i>0.037</i>	0.12
101-9-003744	X	X	4	Draney Creek	10/30/99	F	X		72	75	<b>0.29</b>	1.0	<b>0.10</b>	0.39	<b>0.14</b>	0.51	<i>0.060</i>	0.24
101-9-003781	X	X	5	Rattlesnake Canyon	11/6/99	F		X	72	70	<b>0.34</b>	1.2	<b>0.11</b>	0.36	<b>0.13</b>	0.47	<i>0.049</i>	0.16
101-9-003836	X	X	6	Dry Valley	10/27/99	M		X	70	72	<b>0.98</b>	3.3	<b>0.22</b>	0.78	<b>0.66</b>	2.2	<b>0.075</b>	0.27
101-9-005127	X	X	7	South Sulpher Canyon	11/6/99	M		X	67	70	<b>0.50</b>	1.5	<b>0.21</b>	0.69	<b>0.65</b>	2.0	<b>0.099</b>	0.33
101-9-007510	X	X	8	Sage Creek	10/26/99	F		X	70	73	<b>1.3</b>	4.3	<b>0.12</b>	0.43	<b>0.31</b>	1.0	<b>0.16</b>	0.58
101-9-008142	X	X	9	Dry Valley	10/30/99	F		X	73	71	<b>1.3</b>	4.7	<b>0.17</b>	0.59	<b>0.43</b>	1.6	<b>0.074</b>	0.26
101-9-009603	X	X	10	Pruess Creek	10/30/99	M		X	73	72	<b>0.59</b>	2.2	<b>0.14</b>	0.48	<b>0.29</b>	1.1	<i>0.056</i>	0.20
101-9-010800	X	X	11	Long Valley	11/6/99	M		X	73	76	<b>0.26</b>	1.0	<b>0.10</b>	0.41	<b>0.29</b>	1.1	<b>0.071</b>	0.29
101-9-011498	X	X	89	Trout Creek	10/30/99	F		X	74	74	<b>0.49</b>	1.9	<b>0.063</b>	0.24	<b>0.32</b>	1.2	<i>0.050</i>	0.19
101-9-011744	X	X	12	Rassmussen Valley	10/28/99	F	X		71	76	<b>1.9</b>	6.7	<b>0.15</b>	0.64	<b>0.37</b>	1.3	<i>0.029</i>	0.12
101-9-013229	X	X	13	Slug Creek	10/26/99	F		X	73	68	<b>5.3</b>	19	<b>0.37</b>	1.2	<b>0.50</b>	1.8	<b>0.12</b>	0.38
101-9-015568	X	X	14	South Sulpher Canyon	10/27/99	F		X	71	74	<b>0.39</b>	1.4	<b>0.12</b>	0.45	<b>0.65</b>	2.3	<i>0.051</i>	0.19
101-9-016571	X	X	15	Jacknife Creek	10/27/99	F		X	69	75	<b>0.64</b>	2.1	<b>0.14</b>	0.54	<b>0.49</b>	1.6	<i>0.042</i>	0.17
101-9-018227	X	X	16	Kendall Canyon	10/29/99	F		X	73	69	<b>2.0</b>	7.6	<b>0.33</b>	1.1	<b>0.36</b>	1.3	<b>0.068</b>	0.22
101-9-020202	X	X	17	Wooley Range	10/31/99	F		X	72	74	<b>0.41</b>	1.5	<b>0.13</b>	0.48	<b>0.30</b>	1.1	<i>0.036</i>	0.14
101-9-020457	X	X	18	Woodall Spring	10/26/99	M		X	72	76	<b>1.2</b>	4.2	<b>0.15</b>	0.64	<b>0.42</b>	1.5	<b>0.11</b>	0.45
101-9-022067	X	X	19	Hornet Canyon	10/27/99	F		X	72	70	<b>0.54</b>	1.9	<b>0.13</b>	0.42	<b>0.24</b>	0.83	<b>0.067</b>	0.22
101-9-024783	X	X	20	Diamond Flat	10/29/99	M		X	70	77	<b>1.6</b>	5.2	<b>0.43</b>	1.9	<b>0.51</b>	1.7	<i>0.052</i>	0.23
101-9-024889	X	X	21	Hornet Canyon	10/26/99	?		X	68	73	<b>0.28</b>	0.87	<b>0.11</b>	0.40	<b>0.25</b>	0.79	<i>0.048</i>	0.18
101-9-025926	X	X	22	Fox Hills	10/26/99	M		X	69	74	<b>1.1</b>	3.5	<b>0.29</b>	1.1	<b>0.37</b>	1.2	<i>0.052</i>	0.20
101-9-026635	X	X	23	Little Grey Ridge	10/30/99	F		X	70	69	<b>0.22</b>	0.73	<b>0.10</b>	0.32	<b>0.59</b>	2.0	<b>0.15</b>	0.47
101-9-028332	X	X	83	Morgan Meadows	10/30/99	M		X	69	73	<b>0.71</b>	2.3	<b>0.15</b>	0.57	<b>0.35</b>	1.1	<b>0.11</b>	0.40
101-9-028637	X	X	24	Upper Enoch Valley	10/26/99	F		X	71	72	<b>1.2</b>	4.2	<b>0.27</b>	0.97	<b>1.2</b>	4.1	<b>0.13</b>	0.45
101-9-033823	X	X	96	Tincup Mountain	10/29/99	F		X	70	71	<b>0.68</b>	2.3	<b>0.15</b>	0.51	<b>0.34</b>	1.1	<b>0.16</b>	0.57
101-9-034278	X	X	25	Dry Canyon	10/30/99	F		X	73	73	<b>3.2</b>	12	<b>0.24</b>	0.87	<b>0.33</b>	1.2	<b>0.14</b>	0.51
101-9-041577	X	X	26	Upper Dry Valley	11/7/99	F		X	70	72	<b>2.7</b>	9.1	<b>0.21</b>	0.74	<b>0.37</b>	1.2	<b>0.18</b>	0.66
101-9-045262	X	X	27	Tygee Creek	11/6/99	M		X	70	73	<b>4.6</b>	15	<b>0.48</b>	1.8	<b>0.49</b>	1.6	<b>0.28</b>	1.0
101-9-048459	X	X	28	Dry Valley	10/27/99	F		X	70	73	<b>5.3</b>	18	<b>0.79</b>	2.9	<b>0.34</b>	1.1	<b>0.14</b>	0.51
101-9-054687	X	X	29	Trout Creek	10/26/99	M	X		69	73	<b>0.34</b>	1.1	<b>0.12</b>	0.43	<b>1.6</b>	5.2	<b>0.074</b>	0.27
101-9-057366	X	X	30	Dry Canyon	10/30/99	F		X	65	70	<b>3.4</b>	9.7	<b>0.18</b>	0.60	<b>0.60</b>	1.7	<b>0.14</b>	0.46
101-9-064273	X	X	31	Shield Canyon	11/7/99	F		X	72	74	<b>2.6</b>	9.2	<b>0.52</b>	2.0	<b>0.43</b>	1.5	<b>0.066</b>	0.25
101-9-065581	X	X	32	N. Trail Canyon Road	10/27/99	F		X	74	73	<b>2.2</b>	8.6	<b>0.35</b>	1.3	<b>0.35</b>	1.4	<b>0.11</b>	0.40
101-9-066053	X	X	88	Sage Meadows	10/31/99	F	?	?	73	68	<b>2.4</b>	8.8	<b>0.34</b>	1.1	<b>0.39</b>	1.4	<b>0.11</b>	0.34
101-9-067130	X	X	69	Rassmussen Ridge	10/26/99	M		X	68	76	<b>2.0</b>	6.4	<b>0.17</b>	0.72	<b>0.43</b>	1.4	<b>0.15</b>	0.61
101-9-067901	X	X	33	McCoy Creek	10/30/99	M		X	70	72	<b>0.47</b>	1.6	<b>0.093</b>	0.33	<b>0.33</b>	1.1	<i>0.057</i>	0.20
101-9-069187	X	X	34	Dry Valley	10/31/99	M	X		81	75	<b>2.5</b>	13	<b>0.19</b>	0.76	<b>0.21</b>	1.1	<i>0.022</i>	0.087
101-9-069462	X	X	35	Kendall Canyon	11/5/99	F		X	67	69	<b>0.43</b>	1.3	<b>0.15</b>	0.49	<b>1.1</b>	3.3	<b>0.17</b>	0.56
101-9-072990	X	X	36	Bridge Creek	10/30/99	F		X	72	73	<b>1.5</b>	5.3	<b>0.11</b>	0.40	<b>0.37</b>	1.3	<i>0.015</i>	0.057
101-9-075567	X	X	37	Smoky Canyon	10/27/99	M		X	72	73	<b>0.62</b>	2.2	<b>0.20</b>	0.75	<b>0.29</b>	1.0	<i>0.033</i>	0.12
101-9-081257	X	X	38	Jones Canyon	11/5/99	M		X	65	69	<b>0.35</b>	1.0	<b>0.085</b>	0.28	<b>1.7</b>	4.7	<b>0.071</b>	0.23
101-9-081312	X	X	39	Chippy Creek	10/26/99	M		X	71	69	<b>0.51</b>	1.8	<b>0.13</b>	0.41	<b>0.32</b>	1.1	<i>0.040</i>	0.13
101-9-082092	X	X	40	Jones Canyon	10/29/99	M		X	73	72	<b>0.48</b>	1.8	<b>0.14</b>	0.48	<b>0.73</b>	2.7	<i>0.0095</i>	0.034
101-9-082893	X	X	41	Wooley Valley	10/27/99	F	X		72	73	<b>0.79</b>	2.8	<b>0.12</b>	0.43	<b>0.21</b>	0.72	<i>0.0050</i>	0.018
101-9-083966	X	X	42	Burns Creek	10/26/99	M		X	65	64	<b>0.33</b>	0.93	<b>0.085</b>	0.24	<b>0.23</b>	0.65	<i>0.0018</i>	0.0049
102-9-003525	X	X	43	Summit View Campground	10/31/99	M	X		72	73	<b>0.31</b>	1.1	<b>0.12</b>	0.43	<b>0.19</b>	0.67	<b>0.13</b>	0.47
102-9-007374	X	X	44	Dry Valley Ridge	11/6/99	F	?	?	72	74	<b>7.9</b>	28	<b>0.41</b>	1.6	<b>0.33</b>	1.2	<b>0.095</b>	0.37
102-9-008730	X	X	45	Upper Dry Valley	10/27/99	?	X		71	73	<b>2.3</b>	8.0	<b>0.66</b>	2.4	<b>0.76</b>	2.6	<b>0.14</b>	0.51
102-9-009184	X	X	47	Schmid Ridge	10/30/99	M		X	71	68	<b>1.1</b>	3.7	<b>0.16</b>	0.51	<b>0.46</b>	1.6	<b>0.17</b>	0.54
102-9-009310	X	X	46	Left Hand Fork Georgetown Creek	11/6/99	F	X		71	72	<b>1.7</b>	5.7	<b>0.18</b>	0.63	<b>0.15</b>	0.52	<b>0.068</b>	0.24
102-9-010135	X	X	97	Upper Tincup Creek	10/30/99	F		X	70	75	<b>0.36</b>	1.2	<b>0.12</b>	0.47	<b>0.29</b>	1.0	<i>0.0018</i>	0.0070

**APPENDIX H  
1999 ELK TISSUE LABORATORY ANALYSIS RESULTS**

IDFG License Number	Sample Type		Map ID	General Kill Location	Kill Date	Sex	Age		Liver Moisture Content (%)	Muscle Moisture Content (%)	Selenium				Cadmium			
											Liver		Skeletal Muscle		Liver		Skeletal Muscle	
	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)					Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)			Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)				
	Liver	Muscle																
102-9-010277	X	X	55	Timothy Creek	10/26/99	M		X	71	74	0.50	1.7	0.060	0.23	0.23	0.80	-0.0077	-0.030
102-9-010303	X	X	48	Pole Canyon	10/26/99	F		X	72	74	5.8	21	0.34	1.3	0.21	0.77	-0.016	-0.061
102-9-011973	X	X	49	Pedro Creek	11/6/99	F		X	71	73	0.33	1.1	0.076	0.28	0.43	1.5	0.16	0.59
102-9-012019	X	X	50	Lower Valley	10/30/99	M		X	70	74	0.63	2.1	0.14	0.52	0.29	1.0	0.0086	0.033
104-9-001284	X	X	87	Jones Creek	10/29/99	M		X	73	75	0.57	2.1	0.12	0.47	0.25	0.90	0.013	0.051
104-9-004294	X	X	51	Woodall Mountain	11/7/99	M		X	72	73	1.6	5.6	0.92	3.4	0.45	1.6	0.12	0.44
104-9-005505	X	X	52	Snowdrift Mountain	10/26/99	?	X		70	73	5.7	19	0.29	1.1	0.20	0.66	-0.021	-0.077
104-9-006297	X	X	53	Campbell Canyon	10/29/99	M	X		71	73	5.6	19	0.42	1.6	0.20	0.68	0.12	0.44
104-9-006924	X	X	54	Preuss Creek	10/30/99	M		X	72	70	0.32	1.1	0.076	0.25	0.16	0.57	0.099	0.33
104-9-009499	X	X	86	Jones Canyon	10/30/99	M		X	71	76	1.2	4.0	0.14	0.60	0.30	1.0	0.021	0.088
104-9-014257	X	X	56	Fox Hills	11/7/99	F		X	74	69	0.99	3.8	0.54	1.7	0.48	1.8	0.13	0.41
105-9-000606	X	X	57	Dry Valley	10/30/99	F		X	72	73	1.1	3.8	0.21	0.77	0.62	2.2	0.019	0.071
105-9-004447	X	X	58	Long Valley	11/6/99	F		X	70	72	2.7	9.1	0.19	0.70	0.48	1.6	0.17	0.62
105-9-006076	X	X	59	Timothy Creek	10/27/99	F		X	72	75	0.27	1.0	0.072	0.29	0.22	0.80	-0.012	-0.049
105-9-006125	X	X	60	Dry Valley	10/26/99	F		X	67	72	6.3	19	0.26	0.94	0.42	1.3	0.00034	0.0012
106-9-000834	X	X	61	Deer Creek	11/7/99	F		X	68	74	0.88	2.7	0.13	0.48	0.61	1.9	0.12	0.45
106-9-001250	X	X	62	Upper Rasmussen Valley	10/26/99	F		X	68	74	1.9	6.1	0.33	1.3	0.76	2.4	0.010	0.039
106-9-006628	X	X	63	Schmid Ridge	10/26/99	F		X	71	74	0.47	1.6	0.13	0.48	1.5	5.1	0.0053	0.021
106-9-009470	X	X	64	East Hill	10/30/99	F	X		69	75	0.76	2.4	0.18	0.72	0.46	1.5	0.14	0.55
106-9-010309	X	X	65	Bacon Creek	10/27/99	F		X	71	72	0.37	1.3	0.18	0.63	0.21	0.74	0.012	0.043
107-9-001328	X	X	66	Campbell Canyon	10/30/99	M		X	66	74	0.38	1.1	0.16	0.63	0.65	1.9	0.15	0.56
107-9-002807	X	X	67	Jacknife Creek	10/30/99	M		X	72	76	0.30	1.1	0.12	0.49	0.59	2.1	0.035	0.15
107-9-007918	X	X	68	Diamond Gulch	10/26/99	F		X	69	74	0.74	2.4	0.13	0.48	0.90	2.9	0.048	0.18
107-9-008536	X	X	82	Wooley Valley	10/27/99	F		X	69	73	0.37	1.2	0.18	0.67	0.30	1.0	0.075	0.28
108-9-001128	X	X	90	Diamond Gulch	10/30/99	F		X	65	70	0.57	1.6	0.16	0.54	0.65	1.9	0.11	0.36
108-9-001232	X	X	70	North Deer Creek	10/30/99	F	X		72	73	0.61	2.2	0.20	0.74	0.53	1.9	0.024	0.089
108-9-003843	X	X	71	South Fork Creek	10/26/99	F		X	71	69	0.36	1.2	0.14	0.44	0.24	0.84	0.080	0.26
108-9-004804	X	X	72	Wooley Range	10/31/99	F		X	68	72	2.6	8.2	0.15	0.55	0.52	1.6	0.084	0.30
108-9-005208	X	X	73	Jones Canyon	11/6/99	M	X		73	73	0.31	1.2	0.17	0.62	0.30	1.1	0.099	0.37
108-9-005350	X	X	74	Olsen Creek	11/6/99	M		X	71	75	1.2	4.0	0.14	0.54	0.24	0.84	0.12	0.46
108-9-005351	X	X	75	North of Wolf Mountain	10/26/99	F		X	67	76	0.63	1.9	0.12	0.49	0.91	2.8	0.025	0.10
112-9-000623	X	X	81	Dry Canyon	10/26/99	M	X		71	77	3.2	11	0.34	1.5	0.57	2.0	0.059	0.26
125-9-001720	X	X	78	Freeman Ridge	10/26/99	F		X	69	72	0.47	1.5	0.11	0.38	0.32	1.0	0.065	0.23
125-9-002440	X	X	79	Freeman Ridge	10/26/99	F		X	72	70	0.70	2.5	0.19	0.63	0.43	1.5	0.11	0.36
202-9-003457	X	X	84	North Fork Stump Creek	11/6/99	M		X	69	75	0.40	1.3	0.071	0.28	0.39	1.3	0.14	0.55
202-9-005862	X	X	76	Timothy Creek	10/26/99	M		X	67	74	0.51	1.5	0.091	0.35	0.32	1.0	0.097	0.37
202-9-007411	X	X	77	Black Mountain	10/31/99	M		X	69	73	0.21	0.68	0.077	0.29	0.36	1.2	0.074	0.27
202-9-502746	X	X	85	Stump Peak	10/25/99	M		X	70	66	0.28	0.93	0.14	0.40	0.68	2.3	0.079	0.23
202-9-502746		X	85	Stump Peak	10/25/99	M		X		64			0.12	0.32			0.091	0.25
803-9-00600	X	X	80	Crow Creek	10/26/99	M		X	69	73	0.49	1.6	0.12	0.45	0.32	1.0	0.077	0.29
101-9-000540	X		22	Harrington Peak	10/31/99	M		X	71		0.51	1.8			0.29	0.99		
101-9-001509	X		59	Upper Bacon Creek	11/5/99	F			66		0.34	0.99			0.32	0.93		
101-9-006611	X		77	Warm Creek	11/7/99	F		X	71		0.93	3.2			0.69	2.4		
101-9-006617	X		37	Sage Valley	11/4/99	M		X	69		0.38	1.2			0.32	1.0		
101-9-007574	X		92	Dry Ridge	11/9/99	F		X										
102-9-009231	X		71	Corrailsen Creek	nr	F		X	70		0.39	1.3			0.22	0.75		
101-9-009926	X		63	North Fork Tincup Creek	10/29/99	F		X	68		0.55	1.7			0.40	1.2		
101-9-012050	X		83	Horseshoe Spring	11/4/99	F		X	74		0.59	2.3			0.26	0.99		
101-9-018310	X		85	South Side of Red Mountain	10/30/99	M		X	70		0.71	2.4			0.32	1.1		
101-9-018408	X		74	Rock Creek	nr	M		X	71		0.53	1.8			0.33	1.2		
101-9-019555	X		50	Wolf Mountain	10/30/99	M		X	71		0.69	2.4			0.41	1.4		
101-9-020486	X		78	Chain Hat	10/30/99	F		X	71		0.74	2.6			0.23	0.79		

**APPENDIX H  
1999 ELK TISSUE LABORATORY ANALYSIS RESULTS**

IDFG License Number	Sample Type		Map ID	General Kill Location	Kill Date	Sex	Age		Liver Moisture Content (%)	Muscle Moisture Content (%)	Selenium				Cadmium			
											Liver		Skeletal Muscle		Liver		Skeletal Muscle	
	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)					Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)			Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)				
	Liver	Muscle																
101-9-020534	X		76	Maple Canyon	11/2/99	M		X	71		<b>0.47</b>	1.6			<b>0.33</b>	1.2		
101-9-021815	X		27	Smith Creek	10/26/99	F		X	70		<b>0.40</b>	1.3			<b>0.24</b>	0.81		
101-9-021856	X		53	Trail Creek	10/26/99	M		X										
101-9-023207	X		32	Dry Valley	10/28/99	M	X		70		<b>3.8</b>	13			<b>0.43</b>	1.4		
101-9-025256	X		28	Henry Peak	10/26/99	M		X	67		<b>0.38</b>	1.1			<b>0.51</b>	1.5		
101-9-026241	X		23	Upper Spring Creek	10/26/99	M		X										
101-9-026427	X		93	Boundary Ridge	10/27/99	M	X		70		<b>0.35</b>	1.2			<b>0.22</b>	0.75		
101-9-032948	X		14	Black Mountain	10/27/99	F		X	72		<b>0.37</b>	1.3			<b>0.31</b>	1.1		
101-9-035622	X		20	Upper Slug Creek	10/26/99	F		X	74		<b>4.7</b>	18			<b>0.38</b>	1.5		
101-9-040872	X		33	Dry Ridge	10/26/99	M		X	70		<b>1.4</b>	4.5			<b>0.57</b>	1.9		
101-9-041629	X		98	Boundary Ridge	11/6/99	M	X		72		<b>0.26</b>	0.92			<b>0.16</b>	0.57		
101-9-050246	X		46	Bacon Creek	10/27/99	F		X	72		<b>0.29</b>	1.1			<b>0.14</b>	0.49		
101-9-052544	X		42	Trail Creek Warming Hut	10/28/99	M		X	74		<b>0.28</b>	1.1			<b>0.37</b>	1.4		
101-9-055718	X		73	Deer Creek	11/6/99	F		X	69		<b>0.37</b>	1.2			<b>0.25</b>	0.81		
101-9-057289	X		1	Right Fork Deer Creek	11/4/99	M		X	69		<b>0.59</b>	1.9			<b>0.37</b>	1.2		
101-9-059900	X		84	Southwest Side of Red Mountain	10/30/99	M		X										
101-9-059906	X		81	Southwest Side of Red Mountain	10/30/99	F		X	70		<b>0.90</b>	3.0			<b>0.22</b>	0.75		
101-9-059915	X		86	Pegram Creek	11/3/99	F		X										
101-9-060088	X		8	Woodall Mountain	11/7/99	F		X										
101-9-060482	X		80	Southwest Side of Red Mountain	10/30/99	F		X	71		<b>0.43</b>	1.5			<b>0.25</b>	0.87		
101-9-061415	X		48	Dry Valley	10/27/99	F		X	73		<b>5.2</b>	19			<b>0.32</b>	1.2		
101-9-062127	X		87	Dry Ridge	11/4/99	F	?	?	71		<b>0.47</b>	1.6			<b>0.24</b>	0.85		
101-9-062802	X		5	Shield Canyon	11/7/99	F	X		71		<b>1.9</b>	6.6			<b>0.31</b>	1.1		
101-9-063339	X		89	Tincup Creek	11/8/99	M		X	74		<b>0.26</b>	0.99			<b>0.42</b>	1.6		
101-9-069460	X		25	Big Canyon	10/31/99	F		X	72		<b>9.1</b>	33			<b>0.70</b>	2.5		
101-9-072423	X		6	Coyote Creek	11/6/99	M	X		71		<b>0.38</b>	1.3			<b>0.15</b>	0.52		
101-9-073654	X		3	Smoky Canyon	11/5/99	F		X	71		<b>0.41</b>	1.4			<b>0.16</b>	0.55		
101-9-074150	X		41	Meade Peak	10/26/99	M		X	71		<b>0.39</b>	1.4			<b>0.17</b>	0.58		
101-9-074844	X		95	Eagle Creek	10/30/99	M		X	61		<b>0.34</b>	0.86			<b>0.43</b>	1.1		
101-9-075445	X		75	Smoky Canyon	10/28/99	M		X	72		<b>0.34</b>	1.2			<b>0.24</b>	0.87		
101-9-079565	X		26	State Land Creek	10/27/99	F		X										
101-9-079766	X		7	Olsen Creek	11/6/99	F		X										
101-9-082849	X		35	Church Hollow	11/6/99	F		X	68		<b>0.78</b>	2.4			<b>0.17</b>	0.52		
101-9-084603	X		58	Wolf Mountain	10/30/99	F		X	70		<b>4.4</b>	15			<b>0.85</b>	2.8		
102-9-000978	X		52	Dunn Canyon	10/28/99	F	X		74		<b>0.17</b>	0.66			<b>0.15</b>	0.58		
102-9-003510	X		62	North Fork Tincup Creek	10/26/99	?	X		72		<b>0.23</b>	0.82			<b>0.28</b>	1.0		
102-9-005611	X		29	Wooley Range	10/29/99	M		X	64		<b>2.9</b>	8.1			<b>0.66</b>	1.8		
102-9-008618	X		64	Schmid Ridge	10/26/99	F		X	69		<b>3.6</b>	12			<b>0.32</b>	1.0		
102-9-009523	X		94	Boundary Ridge	10/27/99	F		X										
102-9-009568	X		70	Rasmussen Valley	10/31/99	F		X										
102-9-009651	X		38	Dry Valley	10/26/99	F		X	78		<b>3.7</b>	17			<b>0.24</b>	1.1		
102-9-012285	X		67	Schmid Ridge	10/29/99	F		X	74		<b>1.5</b>	5.6			<b>0.46</b>	1.8		
104-9-002910	X		24	Schmid Ridge	10/26/99	F		X										
104-9-007653	X		82	Bloomington Canyon	10/27/99	M		X	71		<b>0.55</b>	1.9			<b>0.20</b>	0.68		
104-9-008160	X		47	Deer Creek	10/29/99	M		X	90		<b>0.37</b>	3.7			<b>0.32</b>	3.2		
104-9-009086	X		30	Middle Dairy	11/3/99	M	X		75		<b>0.31</b>	1.2			<b>0.15</b>	0.60		
104-9-011485	X		54	Blackfoot River Wildlife Area	10/27/99	M		X	86		<b>2.7</b>	19			<b>0.42</b>	3.0		
105-9-009665	X		72	Snowdrift Mountain	?	F		X										
106-9-002279	X		11	Right Fork Tincup Creek	10/27/99	M	X											
106-9-002669	X		40	Schmid Ridge	10/29/99	M	X		72		<b>0.60</b>	2.1			<b>0.36</b>	1.3		
106-9-005439	X		13	Fossil Canyon	10/26/99	F		X										
106-9-007118	X		43	Timothy	10/26/99	F		X	70		<b>0.31</b>	1.0			<b>0.15</b>	0.50		
106-9-009604	X		60	Coyote Creek	11/6/99	F	?	?	72		<b>0.55</b>	2.0			<b>0.22</b>	0.80		

**APPENDIX H  
1999 ELK TISSUE LABORATORY ANALYSIS RESULTS**

IDFG License Number	Sample Type		Map ID	General Kill Location	Kill Date	Sex	Age		Liver Moisture Content (%)	Muscle Moisture Content (%)	Selenium				Cadmium			
											Liver		Skeletal Muscle		Liver		Skeletal Muscle	
	Liver	Muscle					Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)			Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)	Validated Wet-Weight (mg/kg)	Validated Dry-Weight (mg/kg)		
106-9-009855	X		15	Fossil Canyon	10/26/99	F		X	67		<b>1.3</b>	3.8			<b>0.41</b>	1.2		
107-7-61023	X		79	Diamond Creek	10/30/99	F		X	76		<b>0.20</b>	0.84			<b>0.21</b>	0.86		
107-9-002888	X		66	Dry Ridge	10/26/99	M		X	71		<b>3.1</b>	11			<b>0.32</b>	1.1		
107-9-006997	X		36	Rattlesnake Canyon	11/6/99	F		X										
107-9-007770	X		4	Upper Bacon Creek	11/5/99	F	?	?	70		<b>0.26</b>	0.86			<b>0.20</b>	0.66		
108-9-001165	X		34	Schmid Ridge	10/27/99	F		X	72		<b>4.1</b>	15			<b>0.27</b>	0.96		
108-9-001269	X		65	Dry Ridge	10/28/99	M		X	72		<b>1.7</b>	5.9			<b>0.55</b>	2.0		
108-9-004060	X		91	Tincup Creek	11/7/99	F		X	72		<b>3.5</b>	12			<b>0.50</b>	1.8		
108-9-004918	X		88	The Narrows	11/7/99	F		X	72		<b>0.27</b>	0.96			<b>0.37</b>	1.3		
116-9-9108	X		57	Wolf Mountain	10/30/99	F		X	69		<b>2.9</b>	9.4			<b>0.50</b>	1.6		
116-9-9109	X		56	Wolf Mountain	10/30/99	F		X	70		<b>2.8</b>	9.4			<b>0.20</b>	0.66		
125-9-000899	X		10	Upper Sulphur Canyon	10/26/99	F		X	72		<b>0.44</b>	1.6			<b>0.22</b>	0.80		
125-9-001223	X		17	Caribou Guard Station	10/26/99	F	X		71		<b>7.4</b>	25			<b>0.34</b>	1.2		
202-9-003468	X		2	Diamond Flat	10/29/99	M		X	71		<b>0.31</b>	1.1			<b>0.20</b>	0.67		
202-9-003786	X		99	Diamond Flat	10/30/99	M		X	75		<b>0.26</b>	1.0			<b>0.21</b>	0.86		
202-9-007808	X		96	Deer Creek	10/26/99	M		X										
202-9-508864	X		21	Diamond Creek	10/31/99	F		X	71		<b>0.48</b>	1.7			<b>0.11</b>	0.39		
202-9-509115	X		19	Freeman Ridge	10/27/99	M		X										
202-9-509185	X		18	Upper Dry Valley	10/30/99	M		X										
218-9-000273	X		61	Upper Sulphur Canyon	10/26/99	F	X		71		<b>1.2</b>	4.0			<b>0.23</b>	0.80		
302-9-014681	X		16	North Fork Tincup Creek	10/29/99	F		X										
352-9-000257	X		9	Little Grey Ridge	10/26/99	F		X	69		<b>0.25</b>	0.80			<b>0.23</b>	0.75		
803-9-015407	X		12	North Fork Tincup Creek	10/27/99	F		X	70		<b>0.42</b>	1.4			<b>0.32</b>	1.1		

Notes: Data corrected, in the sequence presented here, for lab blanks, lab-standards slope, and matrix-spike slope; mean reported for stations with replicate samples.  
 Bolded values exceed the 95% upper confidence limit of the 95th percentile of blank results (0.0289 mg/kg for selenium and 0.0635 mg/kg for cadmium) and therefore are discernibly different from a blank.  
 Italicized values do not exceed the 95% upper confidence limit of the 95th percentil of blank results (0.0289 mg/kg for selenium and 0.0635 mg/kg for cadmium) and therefore are not discernibly different from a blank.  
 Samples missing moisture content and chemistry data were omitted from analysis by random selection.  
 nr - not reported  
 Blank cells indicate that there was no value reported.

**Appendix H**  
**DATA VALIDATION PARAMETERS FOR ELK LIVER AND MUSCLE SAMPLES**

<b>Analyte</b>	<b>m<sub>L</sub></b>	<b>b<sub>L</sub></b>	<b>m<sub>F</sub></b>	<b>b<sub>F</sub></b>
Se (Liver)	1.041	-0.007202	0.9921	0.000*
Se (Muscle)	1.041	-0.007202	1.049	0.000*
Cd (Liver)	1.073	-0.004455	1.017	0.000*
Cd (Muscle)	1.073	-0.004455	0.9859	0.000*

No equipment blanks; therefore, a default value of 0 is used.

**Appendix H  
CALCULATION OF UTB VALUES FOR 1999 ELK TISSUE DATA**

**Elk Liver**

Analyte	Lab Blanks <sup>1</sup>				Equipment Blanks <sup>2</sup>				F-Test (1-sided; $\alpha = 0.05$ )				t-Test (2-sided; $\alpha = 0.05$ )			UTB ( $\alpha = 0.05$ ; $p = 0.95$ , $n = v_{pooled} + 1$ )			Notes
	n	v	$\bar{x}$	s	n	v	$\bar{x}$	s	F	p	S <sub>pooled</sub>	V <sub>pooled</sub>	t	p	$\bar{x}$ pooled	g'	UTB	Moisture Adjusted UTB	
Se	48	47	0	0.01385												2.083	0.0289	0.099	No Equipment Blanks
Cd	40	39	0	0.02986												2.125	0.0635	0.219	No Equipment Blanks

**Elk Muscle**

Analyte	Lab Blanks <sup>1</sup>				Equipment Blanks <sup>2</sup>				F-Test (1-sided; $\alpha = 0.05$ )				t-Test (2-sided; $\alpha = 0.05$ )			UTB ( $\alpha = 0.05$ ; $p = 0.95$ , $n = v_{pooled} + 1$ )			Notes
	n	v	$\bar{x}$	s	n	v	$\bar{x}$	s	F	p	S <sub>pooled</sub>	V <sub>pooled</sub>	t	p	$\bar{x}$ pooled	g'	UTB	Moisture Adjusted UTB	
Se	48	47	0	0.01385												2.083	0.0289	0.107	No Equipment Blanks
Cd	40	39	0	0.02986												2.125	0.0635	0.235	No Equipment Blanks

Notes: 1) Laboratory-adjusted results.  
2) Laboratory-and-field-adjusted results.

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## Appendix I

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**IMA's and IDFG's Se ELK Liver Data from SE Idaho Se Project (mg/kg,wet)\***

	Control	Impacted
Mean	0.63	1.51
Standard Deviation	0.55	1.99
Sample Size	27	133
Standard Error	0.106	0.173
Assumed Lower Bound	-0.002	-0.002

\*Minimum variance unbiased estimates of the mean and standard deviation; the latter is corrected for small sample bias.

**Zager's Se Elk Liver Data from Idaho (presumably mg/kg, wet)**

As updated by J. Hansen

Zone	Elk City		Lolo	McCall	
Year	1997	1998	1997	1997	1998
<b>Pre-November 1 Harvest</b>					
Mean	1.19	0.682	0.46	0.27	0.491
Standard Deviation <sup>+</sup>	1.38	1.028	0.44	0.34	1.125
Sample Size	90	73	23	21	30
Standard Error <sup>+</sup>	0.145	0.120	0.092	0.074	0.205
Assumed Lower Bound	0	0	0	0	0
<b>Post-November 1 Harvest</b>					
Mean	1.29	0.727	0.58	0.30	0.692
Standard Deviation <sup>+</sup>	1.45	1.072	0.46	0.49	1.669
Sample Size	74	66	9	8	13
Standard Error	0.169	0.132	0.15	0.17	0.463
Assumed Lower Bound	0	0	0	0	0

<sup>+</sup>Presumably unadjusted for small sample bias.

**Non-Zager<sup>\*</sup> Se Elk and Mule Deer Liver Data from Idaho (presumably mg/kg, wet)**

	All Data:	
	IDFG Region 5 Elk	Elk and Deer Combined
Mean	0.45	0.68
Standard Deviation <sup>+</sup>	0.63	5.9
Sample Size	5	445
Standard Error	0.28	0.28
Assumed Lower Bound	0	0

<sup>\*</sup> *Journal of Wildlife Diseases* 33(4), 1997.

<sup>+</sup>Presumably unadjusted for small sample bias.

**Stussy et al. Se Female Elk Liver Data from Oregon (presumable mg/kg, wet)**

Area	Starkey					Dean Cr.			N. Umpqua		
Year	1989	1990	1991	1992	1993	1990	1991	1987	1989	1990	1991
Mean	0.107	0.065	0.101	0.079	0.144	0.291	0.181	1.777	0.944	0.510	0.253
Standard Deviation <sup>+</sup>	0.05	0.04	0.31	0.04	0.04	0.27	0.21	1.06	0.800	0.45	0.19
Sample Size	36	30	41	34	37	71	74	29	27	43	26
Standard Error <sup>+</sup>	0.009	0.008	0.049	0.007	0.006	0.032	0.024	0.196	0.154	0.068	0.037
Assumed Lower Bound	0	0	0	0	0	0	0	0	0	0	0

<sup>+</sup>Presumably unadjusted for small sample bias.

**IMA's and IDFG's Se and Cd Elk Liver and Muscle Data from SE Idaho Se Project (mg/kg, dry)**

	Se				Cd			
	Liver		Muscle		Liver		Muscle	
	Control	Impacted	Control	Impacted	Control	Impacted	Control	Impacted
Mean	2.2	5.3	0.41	0.77	1.34	1.45	0.34	0.44
Standard Deviation	2.0	7.2	0.124	0.51	0.55	0.74	0.25	0.53
Sample Size	27	133	14	78	27	133	14	78
Standard Error	0.38	0.62	0.033	0.058	0.11	0.064	0.067	0.060
Assumed Lower Bound	-0.0070	-0.0070	-0.0070	-0.0070	-0.092	-0.092	-0.092	-0.092

\*Minimum variance unbiased estimates of the mean and standard deviation; the latter is corrected for small sample bias.

PRINCIPAL COMPONENTS ANALYSIS

Data file - D:\My Documents\Business\SE Idaho Se Project\Se Risk Assessment\1999 Elk Data\Elk Data.mvs

Elk Se and Cd '99 Data

Analysing 4 variables x 91 cases

Tolerance of eigenanalysis set at 1E-10

Eigenvalues

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	1.177	0.640	0.220	0.095
Percentage	55.207	30.021	10.334	4.439
Cum. Percentage	55.207	85.227	95.561	100.000

PCA variable loadings

	Axis 1	Axis 2	Axis 3	Axis 4
$\ln([\text{Se}]_{\text{muscle}} - [\text{Se}]_{\text{muscle},\lambda})$	0.441	0.075	0.220	0.867
$\ln([\text{Cd}]_{\text{muscle}} - [\text{Cd}]_{\text{muscle},\lambda})$	0.047	0.969	-0.236	-0.049
$\ln([\text{Se}]_{\text{liver}} - [\text{Se}]_{\text{liver},\lambda})$	0.896	-0.091	-0.105	-0.422
$\ln([\text{Cd}]_{\text{liver}} - [\text{Cd}]_{\text{liver},\lambda})$	0.009	0.215	0.941	-0.262

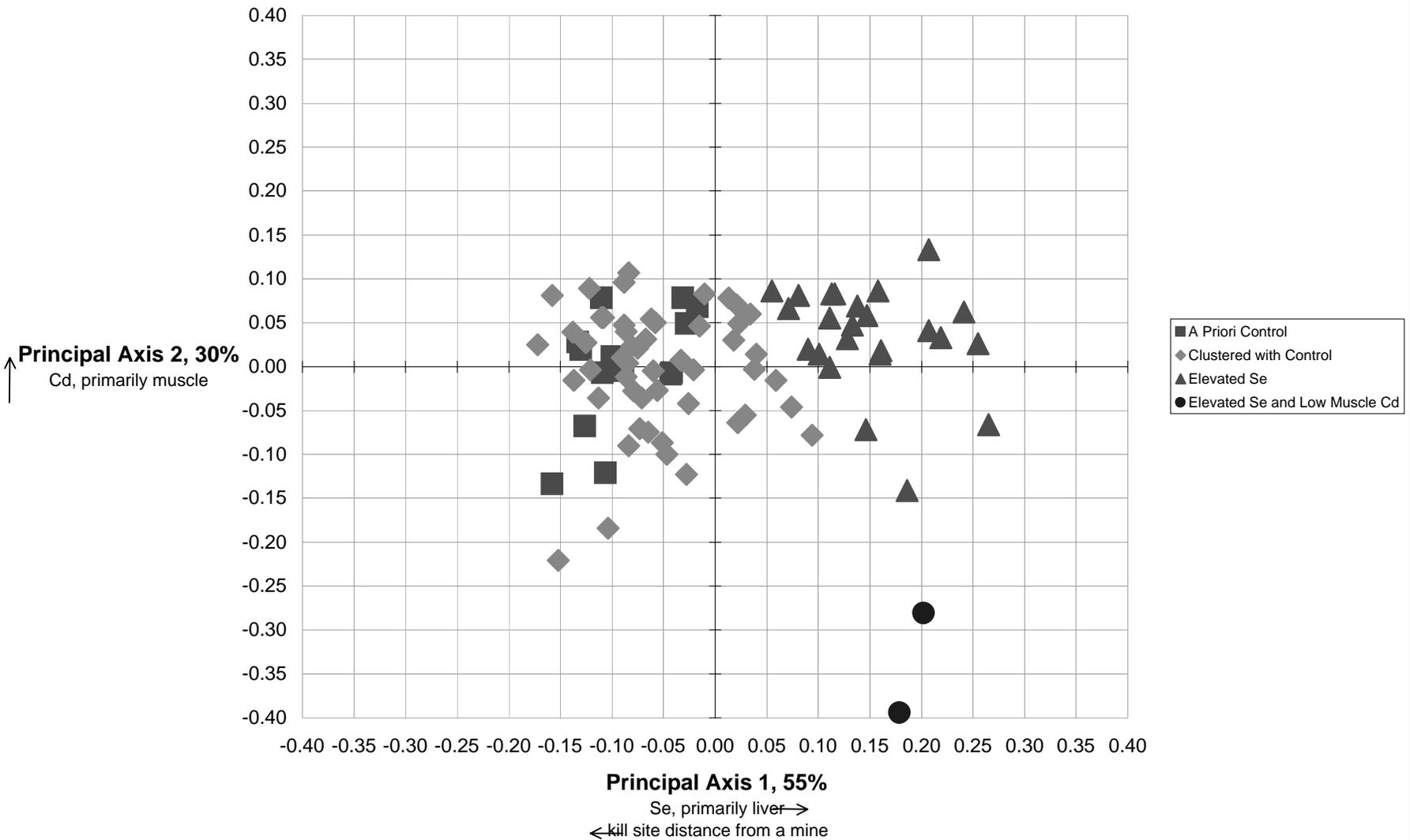
PCA case scores

Elk #	Before-the-Fact Control Elk				Potentially Elevated Elk			
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2	Axis 3	Axis 4
42	-0.158	-0.134	-0.046	-0.007				
85	-0.133	0.028	0.049	-0.010				
54	-0.130	0.019	-0.097	-0.013				
36	-0.126	-0.068	0.019	0.013				
84	-0.110	0.078	-0.033	-0.033				
67	-0.109	-0.007	0.055	0.013				
97	-0.106	-0.121	0.001	0.031				
71	-0.100	0.011	-0.047	0.021				
33	-0.089	-0.005	-0.024	-0.021				
15	-0.043	-0.009	0.020	0.001				
10	-0.042	-0.007	-0.024	-0.002				
96	-0.031	0.078	-0.036	-0.005				
83	-0.028	0.049	-0.028	0.006				
61	-0.017	0.068	0.014	-0.030				
77					-0.172	0.025	-0.019	0.000
23					-0.158	0.081	0.021	-0.010
59					-0.152	-0.221	-0.001	0.008
38					-0.138	0.039	0.114	-0.055
21					-0.137	-0.016	-0.044	0.030
11					-0.125	0.027	-0.025	0.019
49					-0.122	0.089	-0.018	-0.032
4					-0.121	-0.004	-0.092	0.032
5					-0.113	-0.036	-0.095	0.020
29					-0.110	0.056	0.131	-0.025
43					-0.108	0.056	-0.078	0.028
55					-0.104	-0.184	-0.023	-0.038
14					-0.090	0.010	0.053	-0.007
66					-0.088	0.096	0.025	0.030
73					-0.088	0.047	-0.019	0.045
76					-0.086	0.040	-0.047	-0.014
89					-0.086	-0.012	-0.021	-0.058
78					-0.085	0.004	-0.029	-0.006
35					-0.084	0.107	0.073	-0.011
65					-0.084	-0.090	-0.026	0.058
82					-0.081	0.023	-0.026	0.055

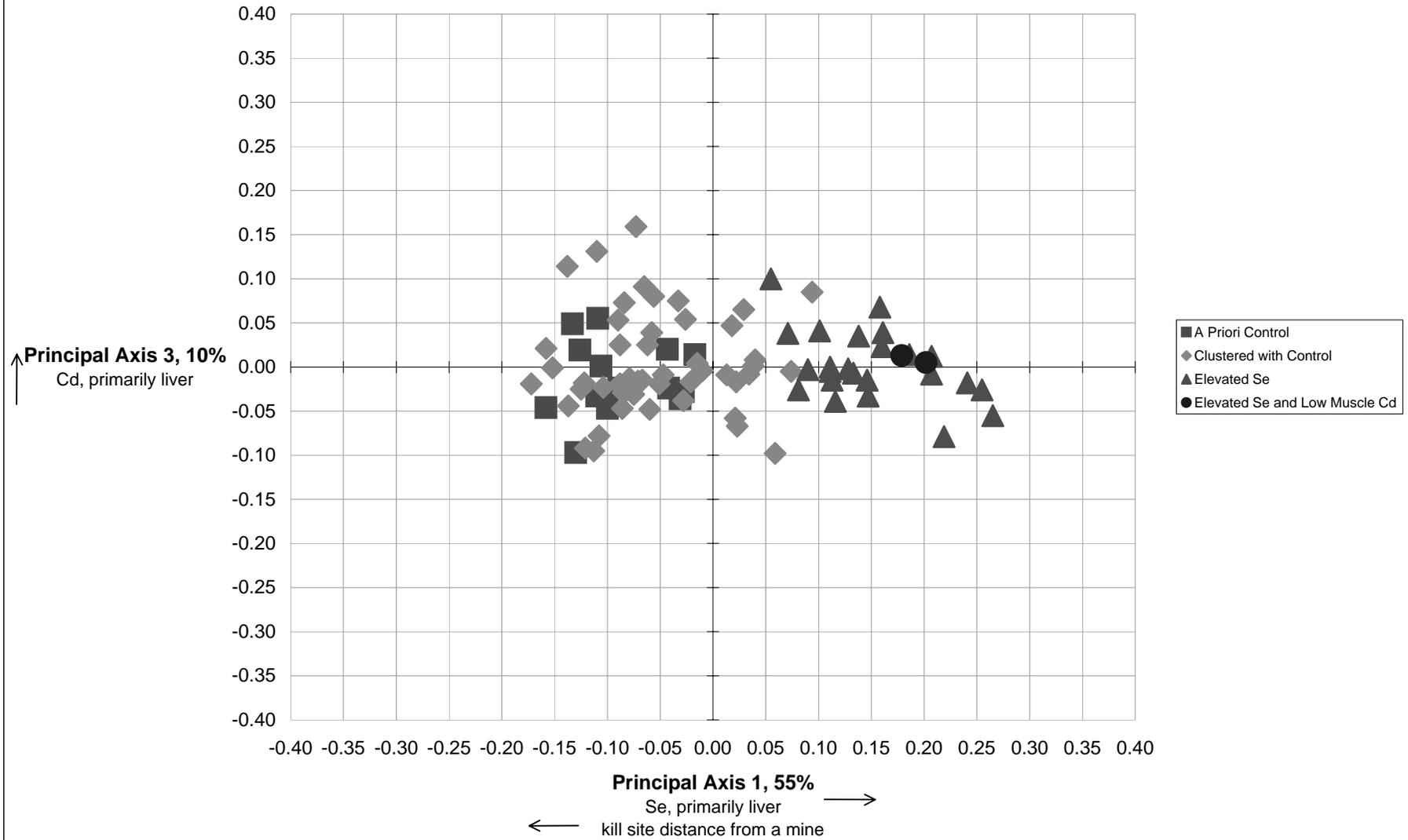
PRINCIPAL COMPONENTS ANALYSIS

Elk #	Before-the-Fact Control Elk				Potentially Elevated Elk			
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2	Axis 3	Axis 4
17					-0.079	-0.028	-0.013	0.017
80					-0.075	0.021	-0.031	0.005
63					-0.073	-0.071	0.159	-0.026
39					-0.071	-0.036	-0.016	-0.006
2					-0.067	0.031	-0.015	-0.005
40					-0.065	-0.075	0.091	-0.014
90					-0.062	0.054	0.025	0.004
19					-0.060	-0.005	-0.048	-0.003
7					-0.058	0.050	0.039	0.028
75					-0.056	-0.027	0.080	-0.018
87					-0.051	-0.087	-0.019	0.004
50					-0.047	-0.100	-0.009	0.014
68					-0.033	0.007	0.075	-0.032
41					-0.028	-0.123	-0.038	-0.008
70					-0.026	-0.042	0.054	0.023
37					-0.021	-0.004	-0.017	0.038
79					-0.015	0.046	0.004	0.004
64					-0.010	0.083	-0.004	0.016
47					0.013	0.078	-0.009	-0.035
6					0.018	0.030	0.047	0.002
8					0.021	0.070	-0.058	-0.044
86					0.022	-0.064	-0.017	-0.006
74					0.023	0.049	-0.067	-0.016
57					0.029	-0.055	0.065	-0.002
18					0.034	0.060	-0.008	-0.018
22					0.038	-0.003	0.001	0.048
9					0.040	0.014	0.008	-0.029
24					0.055	0.086	0.100	-0.008
46					0.059	-0.016	-0.098	-0.003
56					0.071	0.066	0.038	0.070
12					0.074	-0.046	-0.005	-0.030
69					0.081	0.081	-0.026	-0.026
72					0.090	0.020	-0.003	-0.062
62					0.094	-0.078	0.085	0.020
20					0.101	0.014	0.041	0.066
16					0.111	-0.001	0.000	0.006
30					0.111	0.055	-0.005	-0.064
58					0.113	0.083	-0.015	-0.048
26					0.116	0.083	-0.039	-0.035
88					0.128	0.032	-0.002	-0.005
32					0.133	0.047	-0.007	0.015
51					0.138	0.069	0.035	0.118
34					0.146	-0.072	-0.015	-0.041
25					0.147	0.058	-0.033	-0.033
45					0.158	0.086	0.068	0.057
31					0.160	0.014	0.023	0.050
81					0.161	0.018	0.039	0.008
52					0.179	-0.394	0.013	0.005
60					0.186	-0.141	0.014	-0.037
48					0.202	-0.281	0.005	0.008
13					0.207	0.041	0.012	-0.038
27					0.207	0.133	-0.008	0.011
53					0.219	0.033	-0.079	0.014
28					0.241	0.062	-0.018	0.061
44					0.255	0.026	-0.026	-0.016
3					0.265	-0.066	-0.055	-0.040

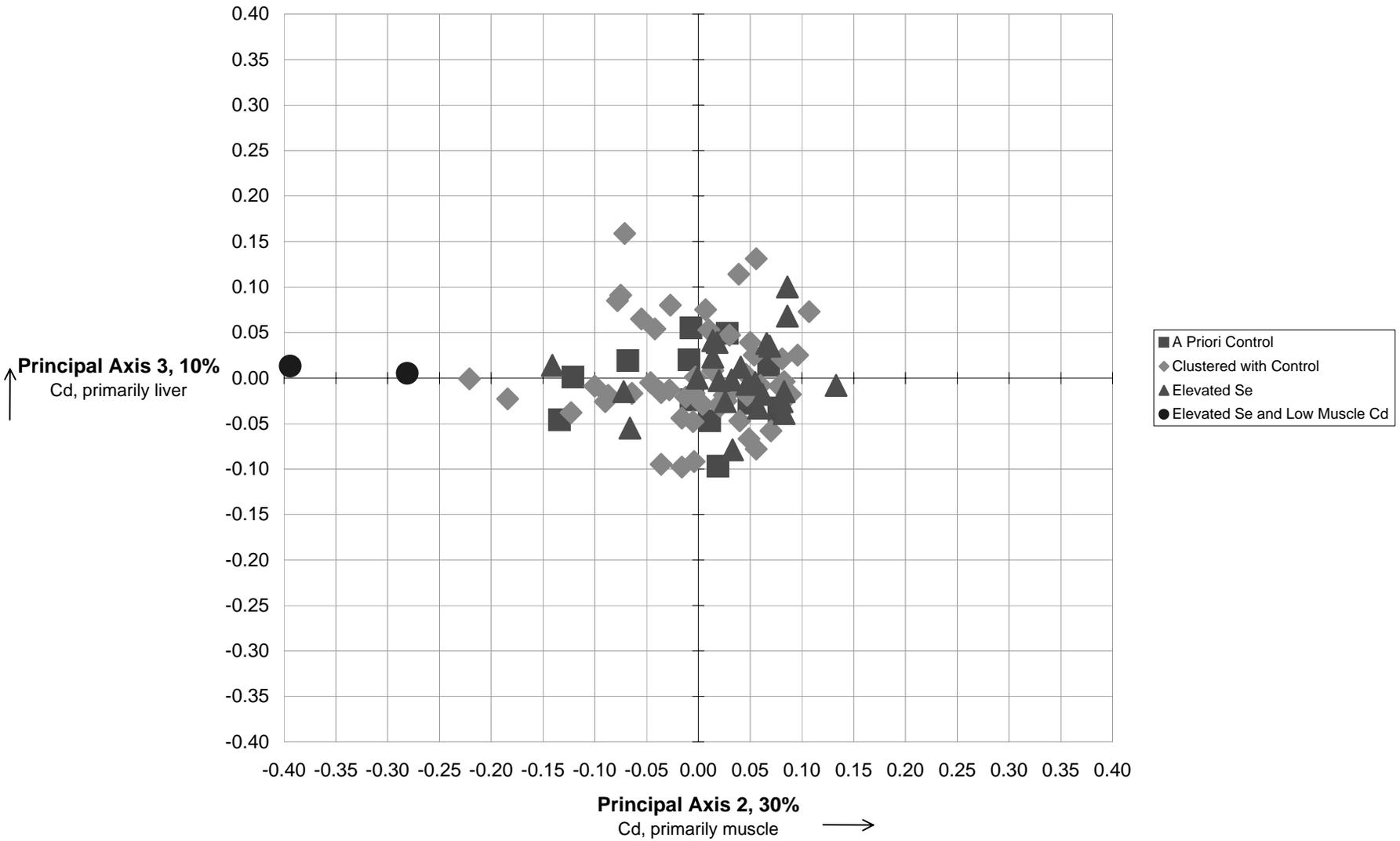
### Principal Components Analysis of Elk Se and Cd, Muscle and Liver, Data



### Principal Components Analysis of Elk Se and Cd, Muscle and Liver, Data



### Principal Components Analysis of Elk Se and Cd, Muscle and Liver, Data



# CLUSTER ANALYSIS

Analysing 4 variables x 91 cases

Minimum variance

Squared Euclidean distance

Result: There are three significant clusters--background, high Se and normal muscle Cd, and high Se and low muscle Cd.

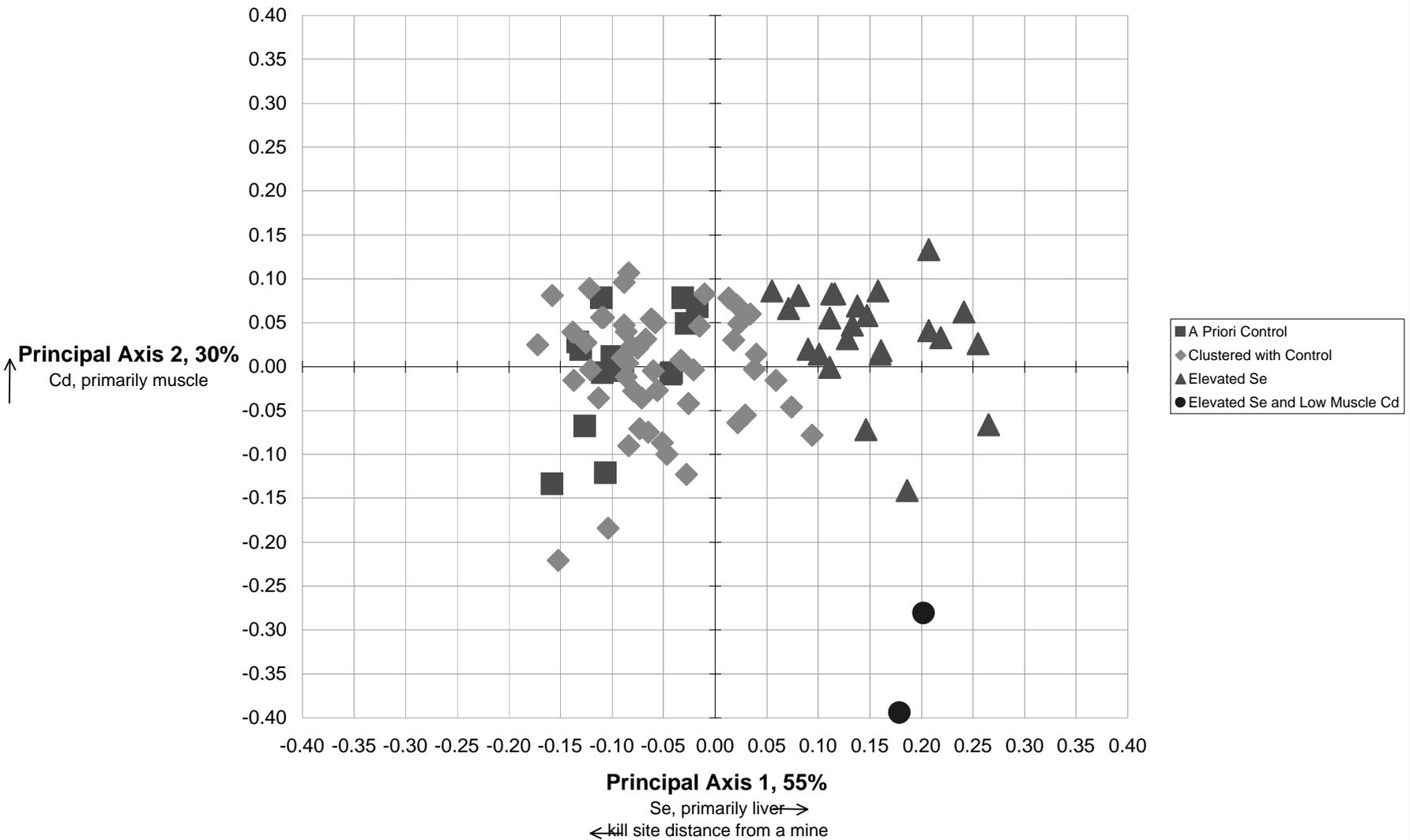
Node	Group 1	Group 2	Sum of squares	Objects in group	$Q_{AB}$	Q	N - g	F	$P_{\text{experimental}}$	c
1	33	78	0.000	2						
2	87	50	0.000	2						
3	84	49	0.000	2						
4	80	2	0.000	2						
5	17	39	0.000	2						
6	88	32	0.000	2						
7	58	26	0.000	2						
8	73	82	0.000	2						
9	90	7	0.000	2						
10	10	19	0.000	2						
11	96	83	0.001	2						
12	67	14	0.001	2						
13	47	18	0.001	2						
14	4	5	0.001	2						
15	8	74	0.001	2						
16	71	11	0.001	2						
17	76	Node 4	0.001	3						
18	79	64	0.001	2						
19	72	30	0.001	2						
20	69	Node 7	0.001	3						
21	75	68	0.001	2						
22	31	81	0.001	2						
23	38	29	0.001	2						
24	61	Node 18	0.001	3						
25	Node 16	21	0.001	3						
26	85	Node 12	0.001	3						
27	16	Node 6	0.001	3						
28	Node 1	Node 17	0.001	5						
29	Node 2	41	0.001	3						
30	97	65	0.001	2						
31	15	70	0.002	2						
32	6	9	0.002	2						
33	Node 10	Node 5	0.002	4						
34	56	20	0.002	2						
35	86	12	0.002	2						
36	Node 20	25	0.002	4						
37	Node 11	Node 24	0.002	5						
38	54	43	0.002	2						
39	37	22	0.002	2						
40	66	Node 9	0.002	3						
41	13	44	0.002	2						
42	63	40	0.002	2						
43	77	23	0.003	2						

# CLUSTER ANALYSIS

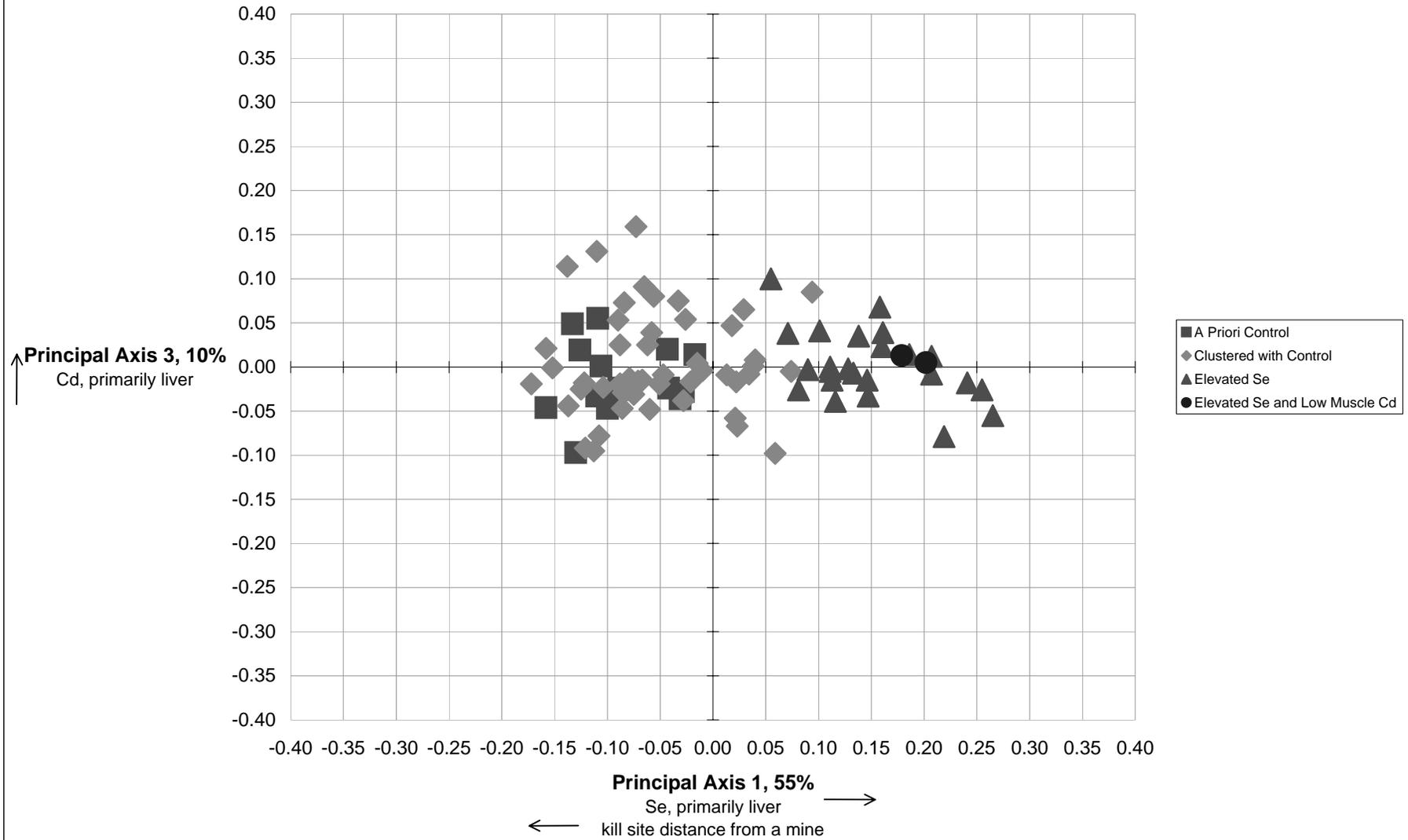
Analysing 4 variables x 91 cases

Node	Group 1	Group 2	Sum of squares	Objects in group	$Q_{AB}$	Q	N - g	F	$P_{\text{experimental}}$	C
44	51	45	0.003	2						
45	57	62	0.003	2						
46	36	Node 30	0.003	3						
47	Node 28	89	0.003	6						
48	Node 13	Node 15	0.003	4						
49	59	55	0.003	2						
50	Node 31	Node 21	0.003	4						
51	Node 40	35	0.004	4						
52	34	60	0.004	2						
53	53	28	0.004	2						
54	Node 25	Node 8	0.004	5						
55	Node 38	Node 14	0.004	4						
56	Node 27	Node 22	0.004	5						
57	Node 36	Node 19	0.004	6						
58	Node 47	Node 33	0.004	10						
59	42	Node 49	0.005	3						
60	Node 3	Node 43	0.005	4						
61	Node 39	Node 32	0.006	4						
62	Node 34	Node 44	0.006	4						
63	Node 41	Node 53	0.006	4						
64	Node 35	46	0.006	3						
65	52	48	0.007	2						
66	Node 37	Node 48	0.007	9						
67	Node 63	27	0.008	5						
68	Node 46	Node 29	0.008	6						
69	Node 55	Node 54	0.009	9						
70	Node 26	Node 23	0.010	5						
71	Node 52	3	0.010	3						
72	Node 50	Node 42	0.012	6						
73	Node 61	Node 64	0.012	7						
74	24	Node 62	0.012	5						
75	Node 70	Node 51	0.015	9						
76	Node 73	Node 45	0.017	9						
77	Node 74	Node 56	0.018	10						
78	Node 69	Node 58	0.018	19						
79	Node 75	Node 60	0.023	13						
80	Node 59	Node 68	0.024	9						
81	Node 77	Node 57	0.038	16						
82	Node 72	Node 76	0.048	15						
83	Node 71	Node 67	0.049	8						
84	Node 82	Node 66	0.069	24						
85	Node 81	Node 83	0.073	24	-0.014	0.087	22	-3.540	#NUM!	
86	Node 79	Node 78	0.073	32						
87	Node 86	Node 84	0.154	56						
88	Node 80	Node 87	0.173	65	-0.005	0.178	63	-1.770	#NUM!	
89	Node 85	Node 65	0.259	26	0.179	0.080	24	53.700	0.000	
90	Node 88	Node 89	0.855	91	0.423	0.432	89	87.146	0.000	4

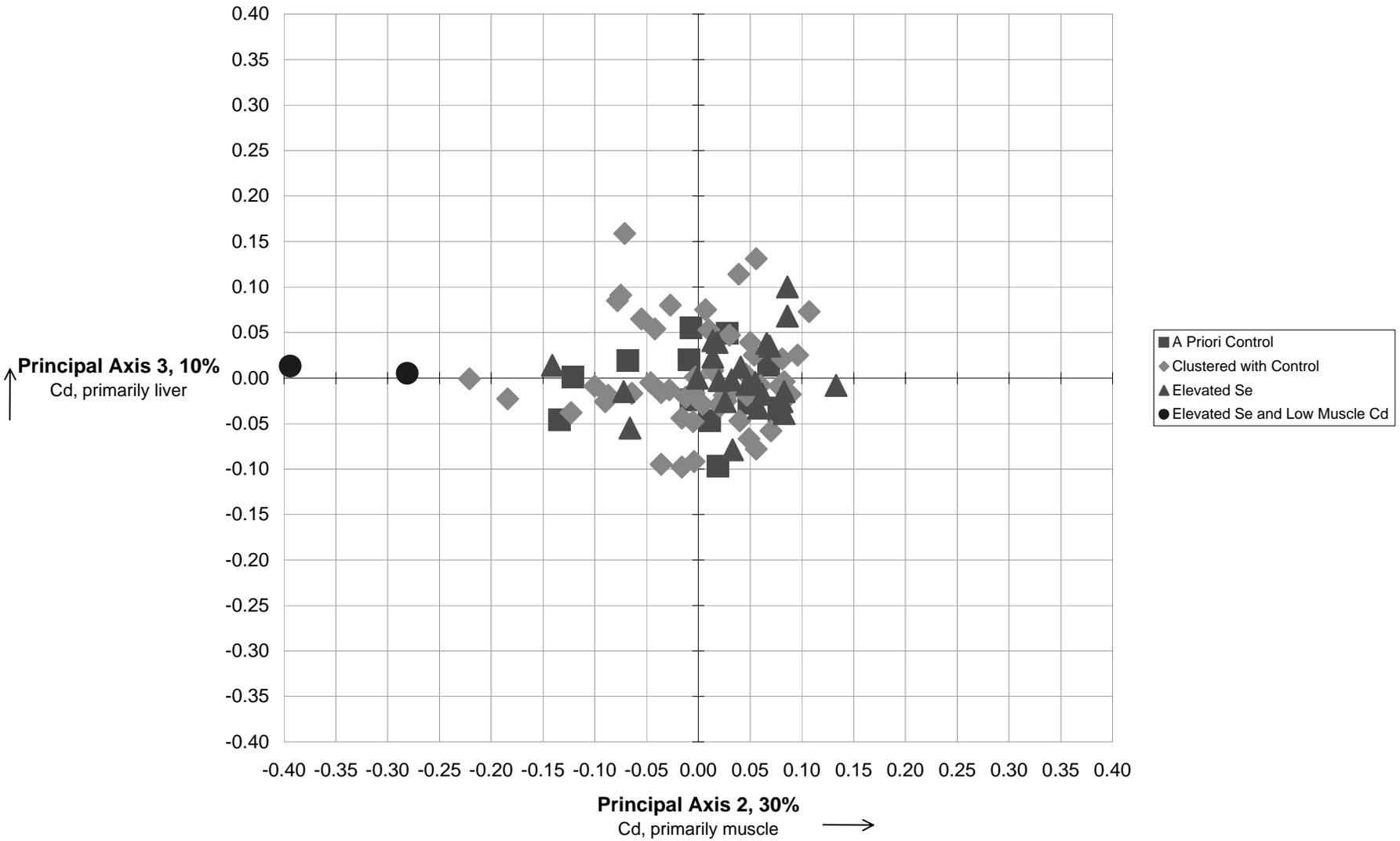
### Principal Components Analysis of Elk Se and Cd, Muscle and Liver, Data



### Principal Components Analysis of Elk Se and Cd, Muscle and Liver, Data



### Principal Components Analysis of Elk Se and Cd, Muscle and Liver, Data



Is liver Se alone sufficient to separate the two functional groups?

Elk#	[Se] <sub>liver</sub> , mg/kg (dry)	ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry	Control Elk	Elk with Elevated Liver Se	Rank	Control Quantile	Elevated Quantile	Control z	Elevated z
			ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry	ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry					
42	0.93	-0.04	-0.35	1.34	1	0.015	0.037	-2.166	-1.786
85	0.93	-0.05	-0.28	1.44	2	0.030	0.074	-1.876	-1.446
54	1.1	0.14	-0.11	1.65	3	0.045	0.111	-1.691	-1.221
36	1.0	0.03	-0.05	1.72	4	0.061	0.148	-1.550	-1.044
84	1.3	0.28	-0.04	1.86	5	0.076	0.185	-1.434	-0.896
67	1.1	0.08	-0.02	2.03	6	0.091	0.222	-1.335	-0.765
97	1.2	0.19	-0.02	2.09	7	0.106	0.259	-1.248	-0.646
71	1.2	0.22	0.01	2.11	8	0.121	0.296	-1.169	-0.535
33	1.6	0.47	0.03	2.15	9	0.136	0.333	-1.097	-0.431
15	2.1	0.73	0.05	2.20	10	0.152	0.370	-1.030	-0.331
10	2.2	0.79	0.08	2.21	11	0.167	0.426	-0.967	-0.187
96	2.3	0.84	0.10	2.21	12	0.182	0.426	-0.908	-0.187
83	2.3	0.84	0.11	2.24	13	0.197	0.481	-0.852	-0.046
61	2.7	1.02	0.14	2.27	14	0.212	0.519	-0.799	0.046
77	0.68	-0.35	0.14	2.40	15	0.227	0.556	-0.748	0.140
23	0.73	-0.28	0.15	2.48	16	0.242	0.593	-0.699	0.234
59	0.96	-0.02	0.16	2.59	17	0.258	0.630	-0.651	0.331
38	0.99	0.01	0.19	2.72	18	0.273	0.667	-0.605	0.431
21	0.87	-0.11	0.20	2.88	19	0.288	0.704	-0.560	0.535
11	0.96	-0.02	0.21	2.94	20	0.303	0.741	-0.516	0.646
49	1.1	0.15	0.22	2.95	21	0.318	0.778	-0.473	0.765
4	1.0	0.05	0.27	2.97	22	0.333	0.815	-0.431	0.896
5	1.2	0.20	0.28	2.98	23	0.348	0.852	-0.389	1.044
29	1.1	0.10	0.28	3.03	24	0.364	0.889	-0.349	1.221
43	1.1	0.11	0.32	3.35	25	0.379	0.926	-0.309	1.446
55	1.7	0.56	0.41	3.64	26	0.394	0.963	-0.269	1.786
14	1.4	0.32	0.43		27	0.409		-0.230	
66	1.1	0.14	0.44		28	0.424		-0.191	
73	1.2	0.16	0.45		29	0.439		-0.153	
76	1.5	0.45	0.47		30	0.455		-0.114	
89	1.9	0.64	0.47		31	0.470		-0.076	
78	1.5	0.44	0.50		32	0.485		-0.038	
35	1.3	0.28	0.50		33	0.500		0.000	
65	1.3	0.27	0.55		34	0.515		0.038	
82	1.2	0.21	0.56		35	0.530		0.076	
17	1.5	0.41	0.58		36	0.545		0.114	
80	1.6	0.47	0.59		37	0.561		0.153	
63	1.6	0.50	0.64		38	0.576		0.191	
39	1.8	0.58	0.65		39	0.591		0.230	
2	1.7	0.55	0.67		40	0.606		0.269	
40	1.8	0.59	0.73		41	0.621		0.309	
90	1.6	0.50	0.75		42	0.636		0.349	
19	1.9	0.67	0.76		43	0.652		0.389	
7	1.5	0.43	0.78		44	0.667		0.431	
75	1.9	0.65	0.79		45	0.682		0.473	
87	2.1	0.76	0.80		46	0.697		0.516	
50	2.1	0.75	0.84		47	0.712		0.560	
68	2.4	0.88	0.84		48	0.727		0.605	
41	2.8	1.05	0.88		49	0.742		0.651	

Is liver Se alone sufficient to separate the two functional groups?

Elk#	[Se] <sub>liver</sub> , mg/kg (dry)	ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry	Control Elk	Elk with Elevated Liver Se	Rank	Control Quantile	Elevated Quantile	Control z	Elevated z
			ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry	ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry					
70	2.2	0.78	0.90		50	0.758		0.699	
37	2.2	0.80	0.93		51	0.773		0.748	
79	2.5	0.93	1.02		52	0.788		0.799	
64	2.4	0.90	1.05		53	0.803		0.852	
47	3.7	1.31	1.19		54	0.818		0.908	
6	3.3	1.19	1.25		55	0.833		0.967	
8	4.3	1.47	1.31		56	0.848		1.030	
86	4.0	1.40	1.35		57	0.864		1.097	
74	4.0	1.40	1.40		58.5	0.886		1.207	
57	3.8	1.35	1.40		58.5	0.886		1.207	
18	4.2	1.43	1.43		60	0.909		1.335	
22	3.5	1.25	1.47		61	0.924		1.434	
9	4.7	1.55	1.55		62	0.939		1.550	
46	5.7	1.74	1.74		63	0.955		1.691	
12	6.7	1.91	1.81		64	0.970		1.876	
62	6.1	1.81	1.91		65	0.985		2.166	
24	4.2	1.44							
56	3.8	1.34							
69	6.4	1.86							
72	8.2	2.11							
20	5.2	1.65							
16	7.6	2.03							
30	9.7	2.27							
58	9.1	2.21							
26	9.1	2.21							
88	9.0	2.20							
32	8.6	2.15							
51	5.6	1.72							
34	13	2.59							
25	12	2.48							
45	8.0	2.09							
31	9.4	2.24							
81	11	2.40							
60	19	2.95							
13	20	2.98							
27	15	2.72							
53	19	2.97							
28	18	2.88							
44	28	3.35							
3	38	3.64							
52	19	2.94							
48	21	3.03							

SUMMARY

Groups	Count	Sum	Average	Variance	F	p
Control	65	38.882	0.598	0.288	1.165	0.31
Elevated Liver Se	26	62.436	2.401	0.335		

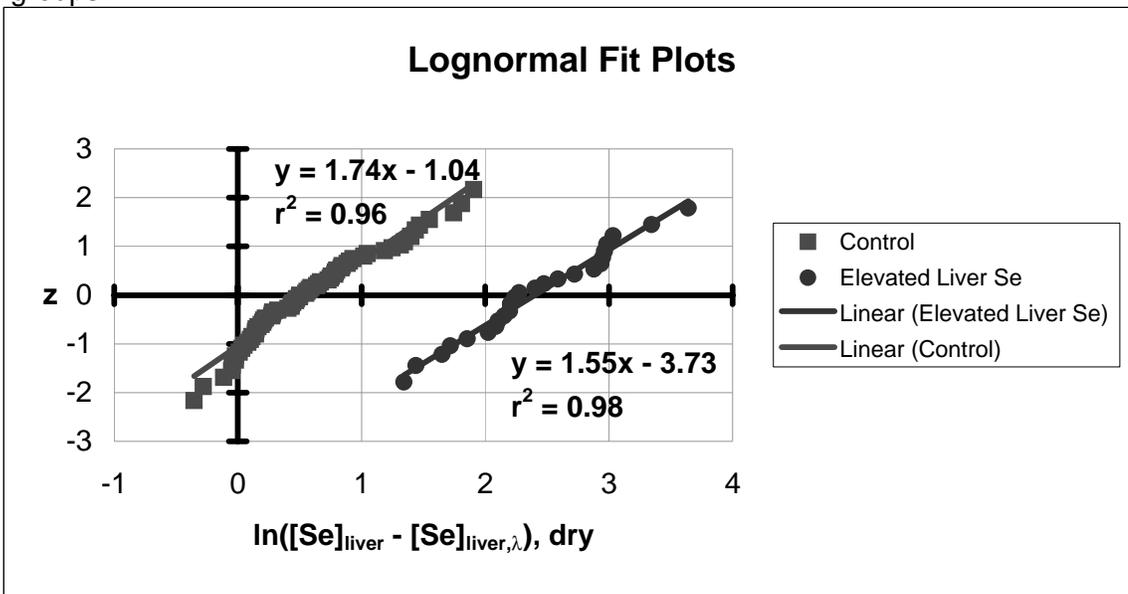
ANOVA

Source of Variation	SS	df	MS	F	p
Between Groups	60.386	1	60.386	200.734	1.6E-24
Within Groups	26.774	89	0.301		
Total	87.160	90			

Retain the null hypothesis of equal variances in the two groups and proceed with the ANOVA.

Reject the null hypothesis of equal means in the two groups.

Result: Liver Se is sufficient alone to discriminate between the two groups.



Are classification, sex, age, or distance of kill site from the nearest PO<sub>4</sub> mine correlated with tissue quality?

Cells shaded yellow denote missing data.

Map ID	A Posteriori Classification (0 = control, 1 = elevated)	Sex (0 = ♀)	Age (0 = < 1)	Distance from Kill Site to Nearest PO <sub>4</sub> Mine, mi	Transformed Distance from Kill Site, ln(d + 1)	ln([Se] <sub>muscle</sub> - [Se] <sub>muscle,i</sub> ), dry	ln([Cd] <sub>muscle</sub> - [Cd] <sub>muscle,i</sub> ), dry	ln([Se] <sub>liver</sub> - [Se] <sub>liver,i</sub> ), dry	ln([Cd] <sub>liver</sub> - [Cd] <sub>liver,i</sub> ), dry	PC Axis 1	PC Axis 2	PC Axis 3	PC Axis 4
2	0	0	1	0.36	0.30	-0.76	-0.93	0.55	0.27	-0.067	0.031	-0.015	-0.005
4	0	0	0	1.9	1.05	-0.87	-1.12	0.05	-0.59	-0.121	-0.004	-0.092	0.032
5	0	0	1	3.6	1.52	-0.96	-1.40	0.20	-0.65	-0.113	-0.036	-0.095	0.020
6	0	1	1	0.80	0.59	-0.22	-1.04	1.19	0.81	0.018	0.030	0.047	0.002
7	0	1	1	4.4	1.68	-0.33	-0.88	0.43	0.71	-0.058	0.050	0.039	0.028
8	0	0	1	0.36	0.30	-0.78	-0.41	1.47	0.07	0.021	0.070	-0.058	-0.044
9	0	0	1	1.3	0.85	-0.48	-1.08	1.55	0.51	0.040	0.014	0.008	-0.029
10	0	1	1	9.1	2.31	-0.68	-1.25	0.79	0.11	-0.042	-0.007	-0.024	-0.002
11	0	1	1	1.7	0.99	-0.83	-0.98	-0.02	0.11	-0.125	0.027	-0.025	0.019
12	0	0	0	0.27	0.24	-0.41	-1.58	1.91	0.28	0.074	-0.046	-0.005	-0.030
14	0	0	1	4.3	1.66	-0.75	-1.28	0.32	0.84	-0.090	0.010	0.053	-0.007
15	0	0	1	17	2.88	-0.57	-1.37	0.73	0.49	-0.043	-0.009	0.020	0.001
17	0	0	1	0.36	0.30	-0.67	-1.49	0.41	0.11	-0.079	-0.028	-0.013	0.017
18	0	1	1	2.1	1.14	-0.41	-0.62	1.43	0.43	0.034	0.060	-0.008	-0.018
19	0	0	1	2.0	1.09	-0.81	-1.18	0.67	-0.10	-0.060	-0.005	-0.048	-0.003
21	0	1	1	0.71	0.54	-0.86	-1.34	-0.11	-0.18	-0.137	-0.016	-0.044	0.030
22	0	1	1	0.89	0.64	0.12	-1.25	1.25	0.22	0.038	-0.003	0.001	0.048
23	0	0	1	4.0	1.61	-1.07	-0.59	-0.28	0.70	-0.158	0.081	0.021	-0.010
29	0	1	0	3.1	1.42	-0.78	-1.03	0.10	1.67	-0.110	0.056	0.131	-0.025
33	0	1	1			-1.03	-1.24	0.47	0.15	-0.089	-0.005	-0.024	-0.021
35	0	0	1	0.6	0.48	-0.65	-0.43	0.28	1.22	-0.084	0.107	0.073	-0.011
36	0	0	1	7	2.03	-0.86	-1.95	0.03	0.32	-0.126	-0.068	0.019	0.013
37	0	1	1	0	0.00	-0.25	-1.24	0.80	0.08	-0.021	-0.004	-0.017	0.038
38	0	1	1	4.6	1.73	-1.20	-1.15	0.01	1.56	-0.138	0.039	0.114	-0.055
39	0	1	1	4.1	1.63	-0.84	-1.54	0.58	0.13	-0.071	-0.036	-0.016	-0.006
40	0	1	1	5.6	1.89	-0.68	-2.13	0.59	1.02	-0.065	-0.075	0.091	-0.014
41	0	0	0	0.71	0.54	-0.78	-2.27	1.05	-0.24	-0.028	-0.123	-0.038	-0.008
42	0	1	1	24	3.20	-1.34	-2.41	-0.04	-0.35	-0.158	-0.134	-0.046	-0.007
43	0	1	0	2.1	1.14	-0.78	-0.59	0.11	-0.33	-0.108	0.056	-0.078	0.028
46	0	0	0	3.2	1.44	-0.42	-1.12	1.74	-0.56	0.059	-0.016	-0.098	-0.003
47	0	1	1	0.62	0.48	-0.63	-0.46	1.31	0.50	0.013	0.078	-0.009	-0.035
49	0	0	1	0.18	0.16	-1.18	-0.40	0.15	0.42	-0.122	0.089	-0.018	-0.032
50	0	1	1	2.4	1.23	-0.61	-2.14	0.75	0.01	-0.047	-0.100	-0.009	0.014
54	0	1	1	6.8	2.05	-1.27	-0.88	0.14	-0.47	-0.130	0.019	-0.097	-0.013
55	0	1	1	2.9	1.37	-1.36	-2.89	0.56	-0.16	-0.104	-0.184	-0.023	-0.038
57	0	0	1	0.71	0.54	-0.23	-1.86	1.35	0.81	0.029	-0.055	0.065	-0.002
59	0	0	1	3.1	1.42	-1.16	-3.32	-0.02	-0.16	-0.152	-0.221	-0.001	0.008
61	0	0	1	14	2.70	-0.67	-0.62	1.02	0.67	-0.017	0.068	0.014	-0.030
62	0	0	1	0.089	0.09	0.25	-2.09	1.81	0.89	0.094	-0.078	0.085	0.020
63	0	0	1	0.53	0.43	-0.67	-2.25	0.50	1.66	-0.073	-0.071	0.159	-0.026
64	0	0	0	2.5	1.25	-0.29	-0.46	0.90	0.43	-0.010	0.083	-0.004	0.016
65	0	0	1	3.1	1.42	-0.42	-2.05	0.27	-0.23	-0.084	-0.090	-0.026	0.058
66	0	1	1			-0.43	-0.44	0.14	0.67	-0.088	0.096	0.025	0.030
67	0	1	1	14	2.71	-0.67	-1.46	0.08	0.77	-0.109	-0.007	0.055	0.013
68	0	0	1	5.7	1.90	-0.67	-1.32	0.88	1.09	-0.033	0.007	0.075	-0.032
70	0	0	0	2.3	1.20	-0.27	-1.75	0.78	0.67	-0.026	-0.042	0.054	0.023
71	0	0	1	10	2.40	-0.77	-1.07	0.22	-0.12	-0.100	0.011	-0.047	0.021
73	0	1	0	4.6	1.73	-0.44	-0.80	0.16	0.14	-0.088	0.047	-0.019	0.045
74	0	1	1	0.98	0.68	-0.57	-0.60	1.40	-0.12	0.023	0.049	-0.067	-0.016
75	0	0	1	2.9	1.37	-0.67	-1.66	0.65	1.03	-0.056	-0.027	0.080	-0.018
76	0	1	1			-0.98	-0.78	0.45	0.02	-0.086	0.040	-0.047	-0.014
77	0	1	1	23	3.17	-1.17	-1.02	-0.35	0.20	-0.172	0.025	-0.019	0.000
78	0	0	1	2.4	1.23	-0.89	-1.15	0.44	0.09	-0.085	0.004	-0.029	-0.006
79	0	0	1	2.1	1.14	-0.42	-0.81	0.93	0.45	-0.015	0.046	0.004	0.004
80	0	1	1	1.1	0.73	-0.75	-0.99	0.47	0.08	-0.075	0.021	-0.031	0.005
82	0	0	1	0.71	0.54	-0.36	-1.01	0.21	0.01	-0.081	0.023	-0.026	0.055
83	0	1	1	12	2.60	-0.52	-0.72	0.84	0.17	-0.028	0.049	-0.028	0.006
84	0	1	1	7.7	2.16	-1.18	-0.46	0.28	0.27	-0.110	0.078	-0.033	-0.033
85	0	1	1	7.5	2.14	-0.95	-1.12	-0.05	0.84	-0.133	0.028	0.049	-0.010
86	0	1	1	7.0	2.07	-0.47	-1.76	1.40	0.07	0.022	-0.064	-0.017	-0.006
87	0	1	1	5.3	1.83	-0.71	-2.00	0.76	-0.03	-0.051	-0.087	-0.019	0.004
89	0	0	1	2.9	1.35	-1.32	-1.29	0.64	0.26	-0.086	-0.012	-0.021	-0.058
90	0	0	1	6.7	2.04	-0.57	-0.81	0.50	0.65	-0.062	0.054	0.025	0.004
96	0	0	1	12	2.53	-0.62	-0.43	0.84	0.18	-0.031	0.078	-0.036	-0.005
97	0	0	1	12	2.60	-0.71	-2.39	0.19	0.01	-0.106	-0.121	0.001	0.031
3	1	0	1	3.0	1.39	0.19	-1.56	3.64	-0.17	0.265	-0.066	-0.055	-0.040
13	1	0	1	1.1	0.73	0.18	-0.76	2.98	0.64	0.207	0.041	0.012	-0.038
16	1	0	1	1.7	0.99	0.08	-1.18	2.03	0.33	0.111	-0.001	0.000	0.006
20	1	1	1	5.4	1.86	0.63	-1.17	1.65	0.57	0.101	0.014	0.041	0.066

Are classification, sex, age, or distance of kill site from the nearest PO<sub>4</sub> mine correlated with tissue quality?

Cells shaded yellow denote missing data.

Map ID	A Posteriori Classification (0 = control, 1 = elevated)	Sex (0 = ♀)	Age (0 = < 1)	Distance from Kill Site to Nearest PO <sub>4</sub> Mine, mi	Transformed Distance from Kill Site, ln(d + 1)	ln([Se] <sub>muscle</sub> - [Se] <sub>muscle,λ</sub> ), dry	ln([Cd] <sub>muscle</sub> - [Cd] <sub>muscle,λ</sub> ), dry	ln([Se] <sub>liver</sub> - [Se] <sub>liver,λ</sub> ), dry	ln([Cd] <sub>liver</sub> - [Cd] <sub>liver,λ</sub> ), dry	PC Axis 1	PC Axis 2	PC Axis 3	PC Axis 4
24	1	0	1	0.089	0.09	0.00	-0.62	1.44	1.43	0.055	0.086	0.100	-0.008
25	1	0	1	0.27	0.24	-0.11	-0.53	2.48	0.25	0.147	0.058	-0.033	-0.033
26	1	0	1	0.89	0.64	-0.26	-0.30	2.21	0.25	0.116	0.083	-0.039	-0.035
27	1	1	1	4.0	1.61	0.60	0.11	2.72	0.52	0.207	0.133	-0.008	0.011
28	1	0	1	0.71	0.54	1.08	-0.53	2.88	0.17	0.241	0.062	-0.018	0.061
30	1	0	1	0.27	0.24	-0.46	-0.62	2.27	0.57	0.111	0.055	-0.005	-0.064
31	1	0	1	0	0.00	0.71	-1.09	2.24	0.46	0.160	0.014	0.023	0.050
32	1	0	1	0	0.00	0.27	-0.72	2.15	0.34	0.133	0.047	-0.007	0.015
34	1	1	0	0.62	0.48	-0.24	-1.76	2.59	0.17	0.146	-0.072	-0.015	-0.041
44	1	0		1.1	0.73	0.47	-0.80	3.35	0.22	0.255	0.026	-0.026	-0.016
45	1		0	0.62	0.48	0.90	-0.53	2.09	0.99	0.158	0.086	0.068	0.057
48	1	0	1	1.1	0.73	0.29	-3.73	3.03	-0.20	0.202	-0.281	0.005	0.008
51	1	1	1	0	0.00	1.24	-0.65	1.72	0.51	0.138	0.069	0.035	0.118
52	1		0	0.18	0.16	0.10	-4.79	2.94	-0.35	0.179	-0.394	0.013	0.005
53	1	1	0	3.7	1.56	0.46	-0.65	2.97	-0.32	0.219	0.033	-0.079	0.014
56	1	0	1	0	0.00	0.57	-0.70	1.34	0.64	0.071	0.066	0.038	0.070
58	1	0	1	0.36	0.30	-0.33	-0.35	2.21	0.50	0.113	0.083	-0.015	-0.048
60	1	0	1	0.27	0.24	-0.03	-2.45	2.95	0.28	0.186	-0.141	0.014	-0.037
69	1	1	1	0.18	0.16	-0.30	-0.37	1.86	0.34	0.081	0.081	-0.026	-0.026
72	1	0	1	0.36	0.30	-0.56	-0.96	2.11	0.51	0.090	0.020	-0.003	-0.062
81	1	1	0	1.3	0.85	0.40	-1.07	2.40	0.71	0.161	0.018	0.039	0.008
88	1	0	1	1.2	0.81	0.08	-0.86	2.20	0.41	0.128	0.032	-0.002	-0.005

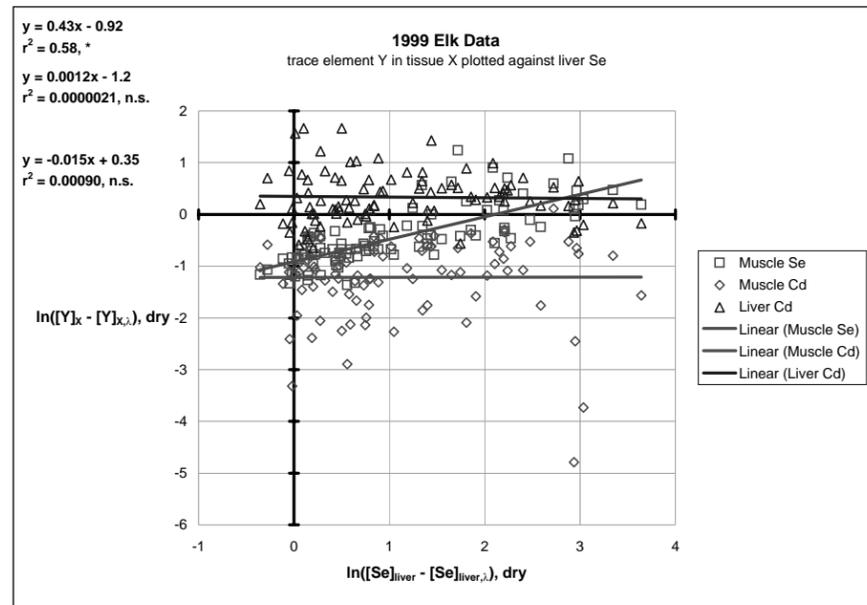
	A Posteriori Classification (0 = control, 1 = elevated)	Sex (0 = ♀)	Age (0 = < 1)	Transformed Distance from Kill Site, ln(d + 1)	ln([Se] <sub>muscle</sub> - [Se] <sub>muscle,i</sub> ), dry	ln([Cd] <sub>muscle</sub> - [Cd] <sub>muscle,i</sub> ), dry	ln([Se] <sub>liver</sub> - [Se] <sub>liver,i</sub> ), dry	ln([Cd] <sub>liver</sub> - [Cd] <sub>liver,i</sub> ), dry	PC Axis 1	PC Axis 2	PC Axis 3	PC Axis 4
A Posteriori Classification (0 = control, 1 = elevated)	1.000											
Sex (0 = ♀)	-0.160	1.000										
Age (0 = < 1)	-0.076	-0.060	1.000									
Transformed Distance from Kill Site, ln(d + 1)	-0.448	0.281	0.101	1.000								
ln([Se] <sub>muscle</sub> - [Se] <sub>muscle,i</sub> ), dry	0.750	-0.108	-0.154	-0.383	1.000							
ln([Cd] <sub>muscle</sub> - [Cd] <sub>muscle,i</sub> ), dry	0.094	0.078	0.094	-0.068	0.127	1.000						
ln([Se] <sub>liver</sub> - [Se] <sub>liver,i</sub> ), dry	0.832	-0.205	-0.146	-0.412	0.763	0.001	1.000					
ln([Cd] <sub>liver</sub> - [Cd] <sub>liver,i</sub> ), dry	0.056	-0.039	0.129	-0.089	0.145	0.229	-0.030	1.000				
PC Axis 1	0.852	-0.189	-0.150	-0.425	0.854	0.065	0.987	0.020	1.000			
PC Axis 2	0.044	0.088	0.114	-0.051	0.108	0.988	-0.073	0.358	0.000	1.000		
PC Axis 3	0.029	-0.046	0.078	-0.068	0.183	-0.140	-0.051	0.918	-0.001	0.000	1.000	
PC Axis 4	0.028	0.113	-0.113	-0.006	0.476	-0.018	-0.131	-0.166	0.001	0.001	0.000	1.000

|r<sub>0.05,experimental</sub>| = 0.291. (n = 84, c = 21.)

Note: Amber-shaded cells are for information only, not for hypothesis testing.

Results:

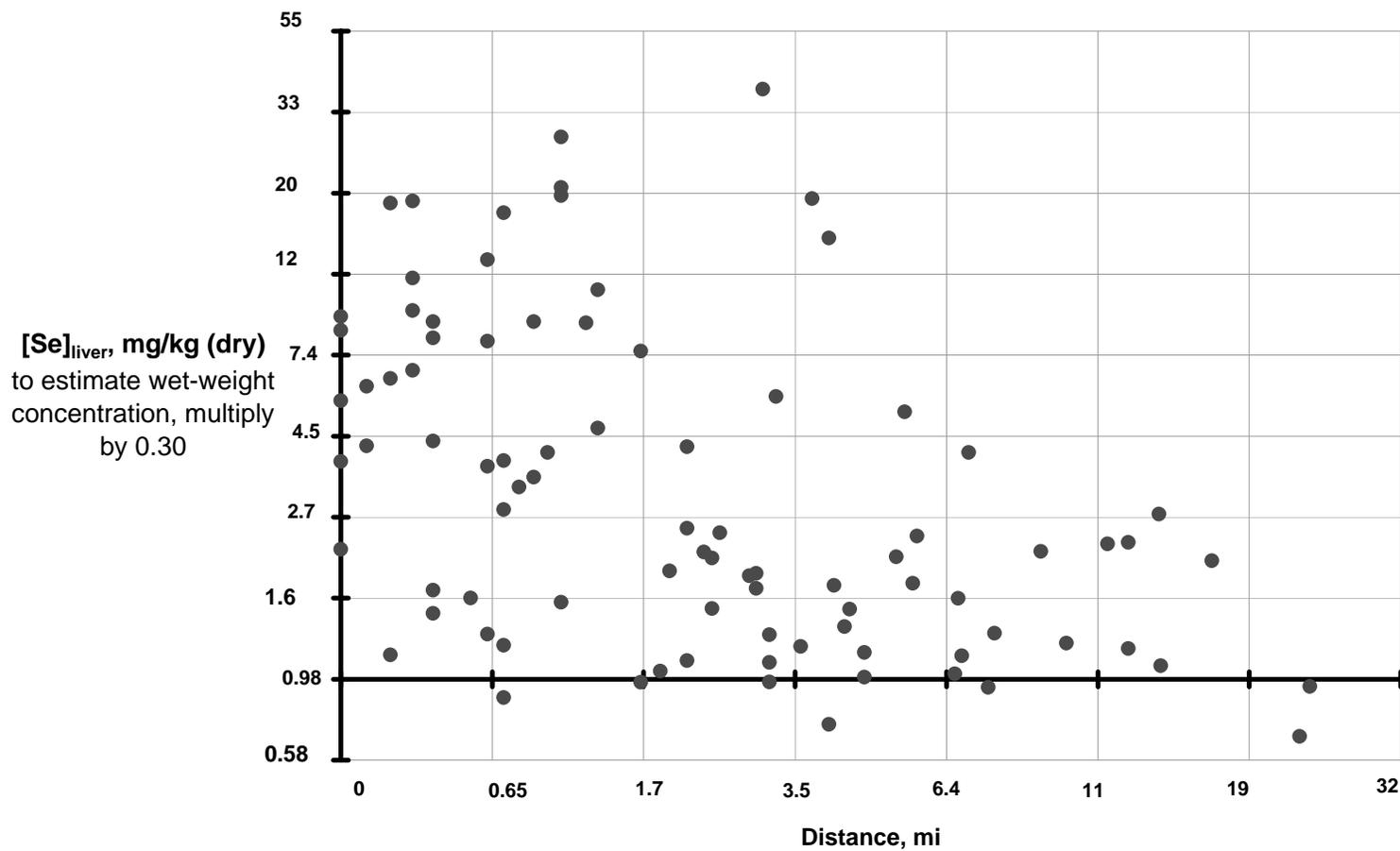
- Kill-site distance is inversely correlated with muscle Se and liver Se (the closer the kill site to a mine, the more likely the elk's tissue is elevated with Se).
- Muscle Se is directly correlated with liver Se (an elk high in muscle Se is likely to be high in liver Se).



<b>Map ID</b>	<b>Distance from Kill Site to Nearest PO<sub>4</sub> Mine, d, mi</b>	<b>ln(d+1)</b>	<b>ln([Se]<sub>liver</sub> - [Se]<sub>liver, λ</sub>), dry</b>
2	0.36	0.30	0.55
4	1.9	1.05	0.05
5	3.6	1.52	0.20
6	0.80	0.59	1.19
7	4.4	1.68	0.43
8	0.36	0.30	1.47
9	1.3	0.85	1.55
10	9.1	2.31	0.79
11	1.7	0.99	-0.02
12	0.27	0.24	1.91
14	4.3	1.66	0.32
15	17	2.88	0.73
17	0.36	0.30	0.41
18	2.1	1.14	1.43
19	2.0	1.09	0.67
21	0.71	0.54	-0.11
22	0.89	0.64	1.25
23	4.0	1.61	-0.28
29	3.1	1.42	0.10
35	0.62	0.48	0.28
36	6.6	2.03	0.03
37	0	0.00	0.80
38	4.6	1.73	0.01
39	4.1	1.63	0.58
40	5.6	1.89	0.59
41	0.71	0.54	1.05
42	24	3.20	-0.04
43	2.1	1.14	0.11
46	3.2	1.44	1.74
47	0.62	0.48	1.31
49	0.18	0.16	0.15
50	2.4	1.23	0.75
54	6.8	2.05	0.14
55	2.9	1.37	0.56
57	0.71	0.54	1.35
59	3.1	1.42	-0.02
61	14	2.70	1.02
62	0.089	0.09	1.81
63	0.53	0.43	0.50
64	2.5	1.25	0.90
65	3.1	1.42	0.27
67	14	2.71	0.08
68	5.7	1.90	0.88
70	2.3	1.20	0.78
71	10	2.40	0.22
73	4.6	1.73	0.16
74	0.98	0.68	1.40
75	2.9	1.37	0.65
77	23	3.17	-0.35
78	2.4	1.23	0.44
79	2.1	1.14	0.93

<b>Map ID</b>	<b>Distance from Kill Site to Nearest PO<sub>4</sub> Mine, d, mi</b>	<b>ln(d+1)</b>	<b>ln([Se]<sub>liver</sub> - [Se]<sub>liver, λ</sub>), dry</b>
80	1.1	0.73	0.47
82	0.71	0.54	0.21
83	12	2.60	0.84
84	7.7	2.16	0.28
85	7.5	2.14	-0.05
86	7.0	2.07	1.40
87	5.3	1.83	0.76
89	2.9	1.35	0.64
90	6.7	2.04	0.50
96	12	2.53	0.84
97	12	2.60	0.19
3	3.0	1.39	3.64
13	1.1	0.73	2.98
16	1.7	0.99	2.03
20	5.4	1.86	1.65
24	0.09	0.09	1.44
25	0.27	0.24	2.48
26	0.89	0.64	2.21
27	4.0	1.61	2.72
28	0.71	0.54	2.88
30	0.27	0.24	2.27
31	0	0.00	2.24
32	0	0.00	2.15
34	0.62	0.48	2.59
44	1.1	0.73	3.35
45	0.62	0.48	2.09
48	1.1	0.73	3.03
51	0	0.00	1.72
52	0.18	0.16	2.94
53	3.7	1.56	2.97
56	0	0.00	1.34
58	0.36	0.30	2.21
60	0.27	0.24	2.95
69	0.18	0.16	1.86
72	0.36	0.30	2.11
81	1.3	0.85	2.40
88	1.2	0.81	2.20

Elk Liver Se as a Function of Kill-Site Distance from a PO<sub>4</sub> Mine



Statistical Analyses for IMA/IDFG Elk Se and Cd Data

ANOVA: Single Factor--[Se] in Elk Liver (using a 3-parameter lognormal transform on the dry-weight data)

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Control	27	13.83	0.51	0.63	1.70	0.058	0.41
Impacted	133	152.07	1.14	1.06			

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>v</i>	<i>MS</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Between Groups	8.94	1	8.94	9.01	0.0031	0.028
Within Groups	156.81	158	0.99			
Total	165.75	159				

Conclusions:

There is no discernible difference ( $p_{\text{experimental}} \geq 0.050$ ) in the variances of the transformed control and impacted data; therefore--

- retain the null hypothesis of homoscedasticity;
- use the within-group MS as an estimate of combined variance; and,
- proceed with the ANOVA.

There is a significant difference ( $p_{\text{experimental}} < 0.050$ ) in the control and impacted data means; therefore--

- reject the null hypothesis of no difference in the means of the transformed concentrations; and,
- conclude that exposure to mining-related Se releases, as estimated by kill distance from a phosphate mine, results in increased liver concentrations.

ANOVA: Single Factor--[Se] in Elk Muscle (using a 3-parameter lognormal transform on the dry-weight data)

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Control	14	-13.15	-0.94	0.08	4.29	0.0028	0.025
Impacted	78	-34.03	-0.44	0.36			

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>v</i>	<i>MS</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Between Groups	3.00	1	3.00	9.26	0.0031	0.027
Within Groups	29.14	90	0.32			
Total	32.14	91				

Conclusions:

- There is a significant difference ( $p_{\text{experimental}} < 0.050$ ) in the variances of the transformed control and impacted data; therefore--
- reject the null hypothesis of homoscedasticity--assume heteroscedasticity;
  - because the within-group MS can not be used as an estimate of combined variance, the above ANOVA is not valid; and,
  - use a  $t'$  test.

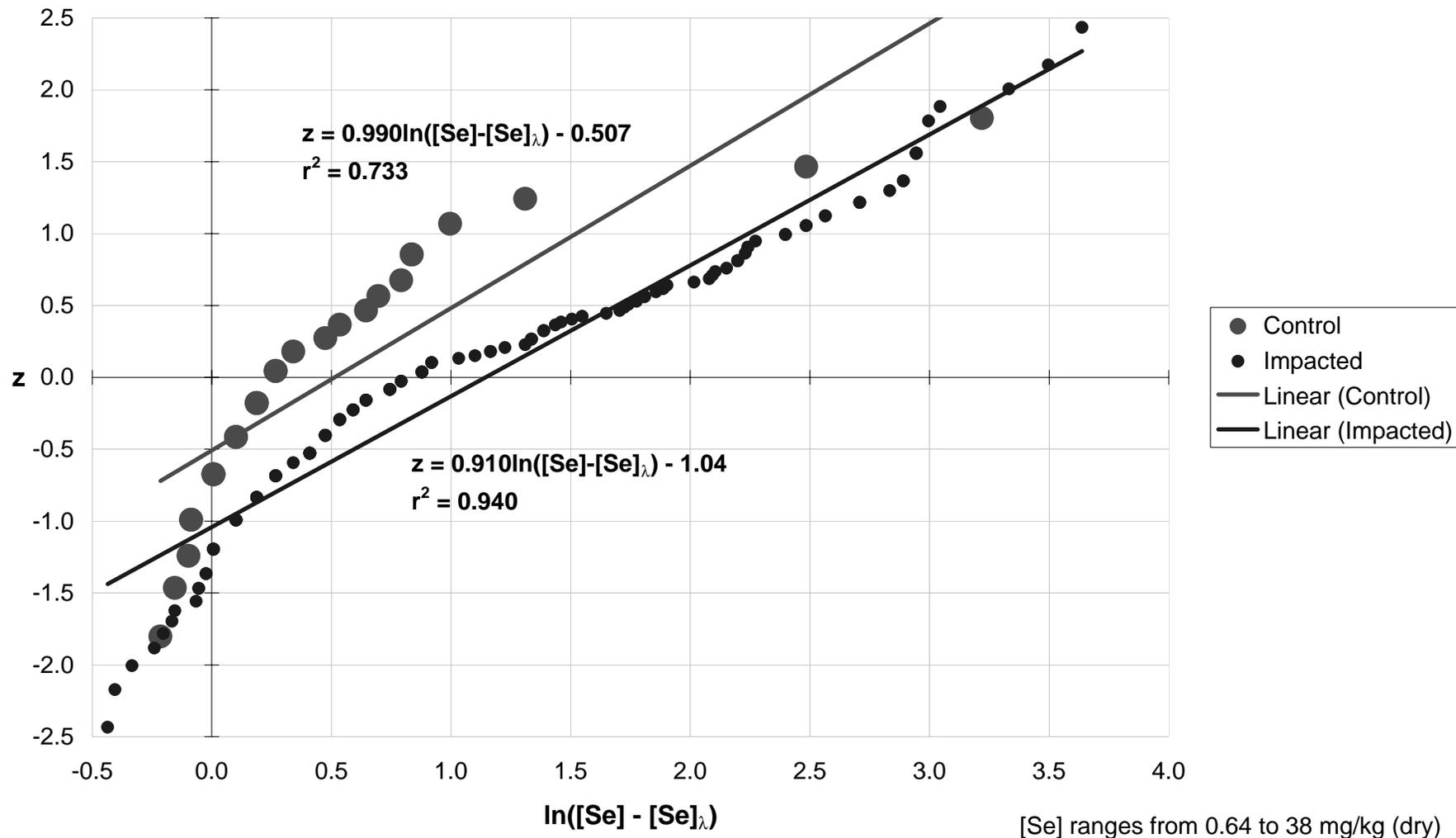
$t'$  Test--[Se] in Elk Muscle (using a 3-parameter lognormal transform on the dry-weight data)

$t'$	4.85
$t'_{\text{critical}}$ (one-sided, $\alpha = 0.050$ )	1.72
$t'_{\text{critical}}$ (one-sided, $\alpha_{\text{experimental}} = 0.050$ )	2.79

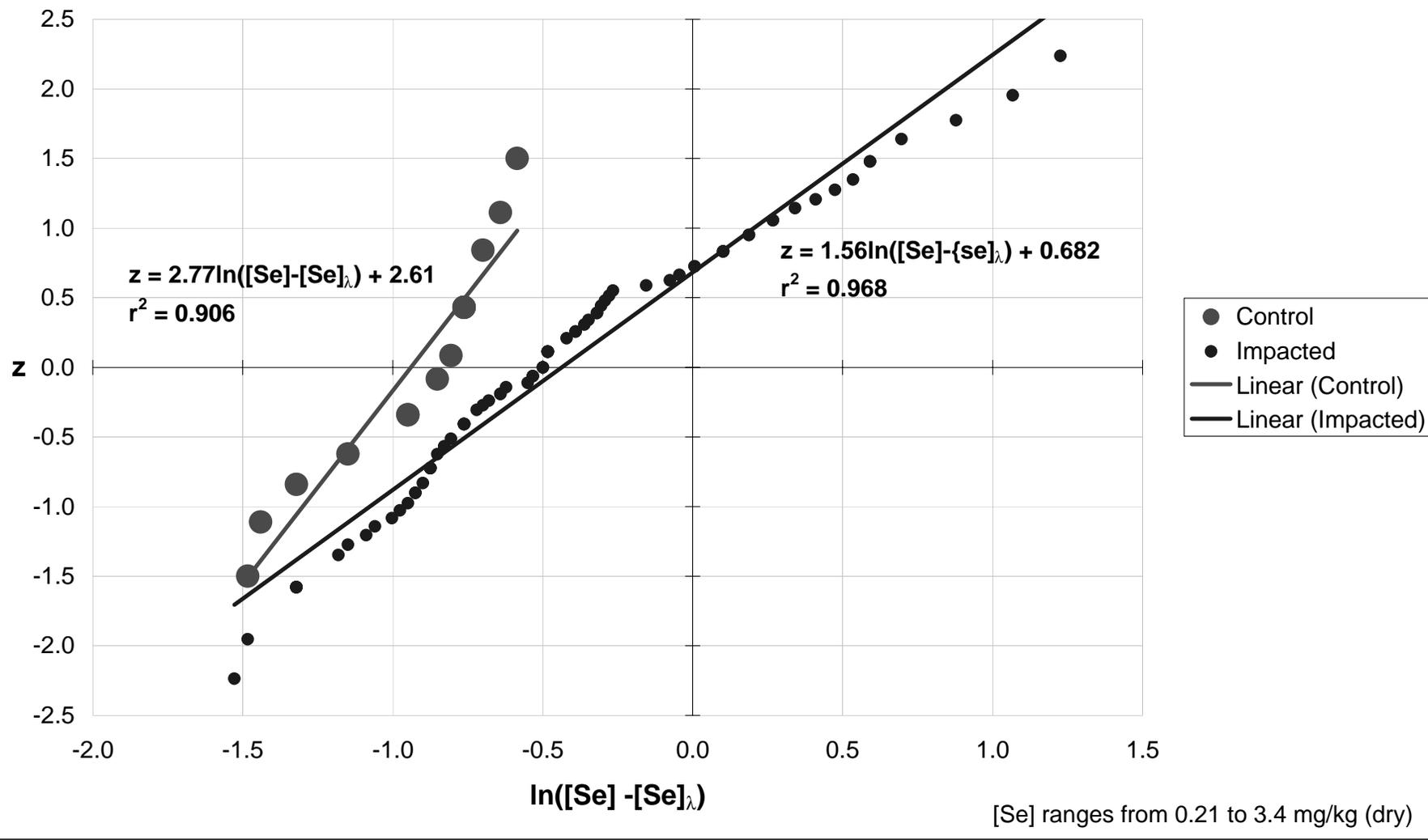
Conclusions:

- The mean of the transformed impacted data is significantly higher ( $p_{\text{experimental}} < 0.050$ ) than that of the transformed control data; therefore--
- reject the null hypothesis of no difference in transformed means; and,
  - conclude that exposure to mining-related Se releases, as estimated by kill distance from a phosphate mine, results in increased muscle concentrations.

### 1999 Elk Data: Se in Liver dry weight



### 1999 Elk Data: Se in Muscle dry weight



ANOVA: Single Factor--[Cd] in Elk Liver (using a 3-parameter lognormal transform on the dry-weight data)

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Control	27	5.74	0.21	0.15	1.50	0.11	0.66
Impacted	133	34.20	0.26	0.23			

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>v</i>	<i>MS</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Between Groups	0.04	1	0.04	0.20	0.65	1.0
Within Groups	34.65	158	0.22			
Total	34.70	159				

Conclusions:

There is no discernible difference ( $p_{\text{experimental}} \geq 0.050$ ) in the variances of the transformed control and impacted data; therefore--

- retain the null hypothesis of homoscedasticity;
- use the within-group MS as an estimate of combined variance; and,
- proceed with the ANOVA.

There is no discernible difference ( $p_{\text{experimental}} \geq 0.050$ ) in the control and impacted data means; therefore--

- retain the null hypothesis of no difference in the means of the transformed concentrations; and,
- conclude that exposure to mining-related Cd releases, as estimated by kill distance from a phosphate mine, results in no increase in liver concentrations.

ANOVA: Single Factor--[Cd] in Elk Muscle (using a 3-parameter lognormal transform on the dry-weight data)

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Control	14	-17.80	-1.27	0.44	2.14	0.064	0.45
Impacted	78	-99.36	-1.27	0.93			

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>v</i>	<i>MS</i>	<i>F</i>	<i>p</i>	<i>p<sub>experimental</sub></i>
Between Groups	0.00	1	0.00	0.00	0.99	1.0
Within Groups	77.50	90	0.86			
Total	77.50	91				

Conclusions:

There is no discernible difference ( $p_{\text{experimental}} \geq 0.050$ ) in the variances of the transformed control and impacted data; therefore--

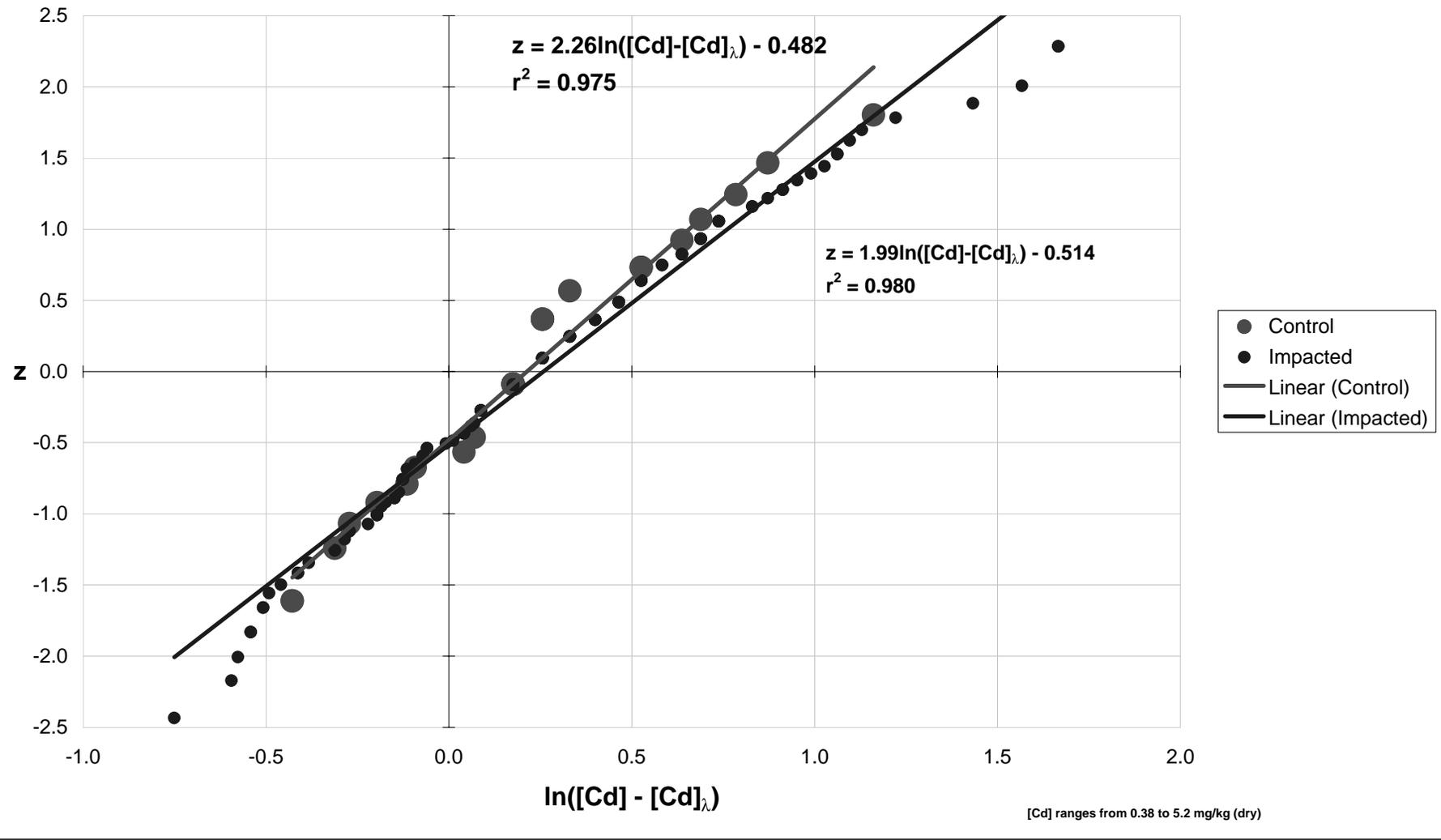
- retain the null hypothesis of homoscedasticity;
- use the within-group MS as an estimate of combined variance; and,
- proceed with the ANOVA.

There is no discernible difference ( $p_{\text{experimental}} \geq 0.050$ ) in the control and impacted data means; therefore--

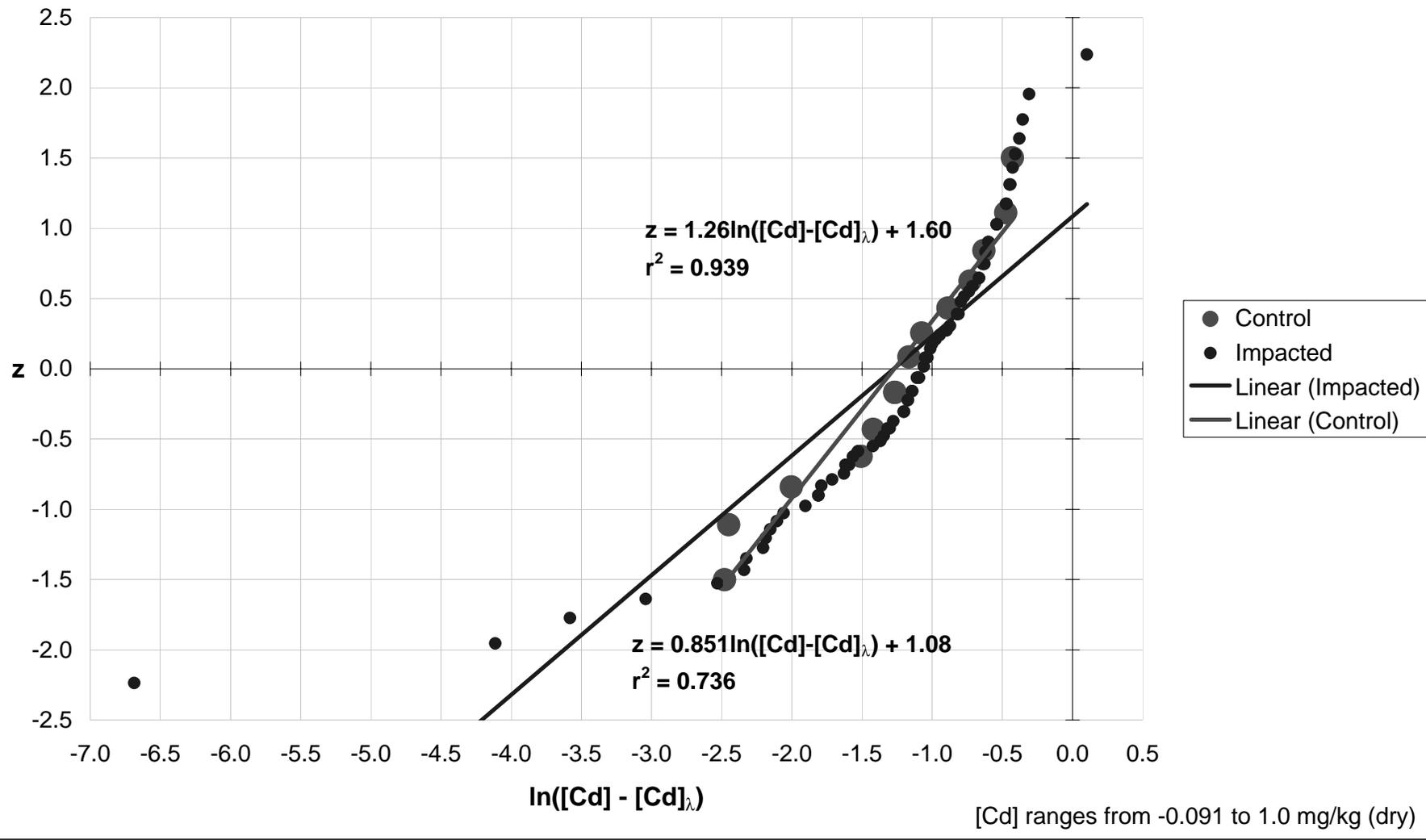
- retain the null hypothesis of no difference in the means of the transformed concentrations; and,
- conclude that exposure to mining-related Cd releases, as estimated by kill distance from a phosphate mine, results in no increase in muscle concentrations.

Note on Type I (false positive) error rates ( $p$  and  $\alpha$ ): In the hypothesis tests above, the traditional Type I error rate of 0.050 is used as the decision criterion for significance. However, a  $p$  value of 0.050 is equal to a Type I error rate 0.050 only if a single test is being performed. In an experiment consisting of multiple tests the experimental Type I error rate,  $p_{\text{experimental}}$ , is calculated as follows:  $p_{\text{experimental}} = 1 - (1 - p)^r$ , where  $p$  is the Type I error rate if only a single test is being performed and  $r$  is the number of tests performed in the experiment. In the above experiment 9 tests are performed--4 F tests, 4 ANOVAs, and 1 t' test; thus,  $r = 9$ . A value of  $\alpha$  needed to attain a specified value for  $\alpha_{\text{experimental}}$  is calculated by substituting  $\alpha_{\text{experimental}}$  for  $p$  in the equation and replacing  $r$  with  $1/r$ .

### 1999 Elk Data: Cd in Liver dry weight



### 1999 Elk Data: Cd in Muscle dry weight



A simple conservative bounding assessment of risk is presented below to demonstrate that elk #51, with the maximum observed liver Se content of 13 mg/kg (wet), is safe to consume on a chronic-consumption basis:

- Assume that one eats 4 oz, or 0.1135 kg, of elk skeletal muscle per day (this is approximately twice the national average beef consumption rate according to the United States Environmental Protection Agency (USEPA, 1996, *Exposure Factors Handbook*, Volumes I, II, and III, EPA/600/8-89-043, USEPA, Washington, District of Columbia).
- Assume a Se skeletal muscle content of 0.36 mg/kg (wet) (the skeletal muscle concentration in elk #51).
- These assumptions equate to a daily Se intake, from elk #51 skeletal muscle, of 0.041 mg, or 41 µg.
- Assume that an elk liver is 5 lb, and that it is consumed over the course of one year—i.e., 0.006219 kg/d.
- Assume a Se liver content of 13 mg/kg (wet) (the liver concentration in elk #51, the maximum such concentration observed).
- The prior two assumptions equate to a maximum daily Se intake, from liver, of 0.0808 mg, or 80.8 µg.
- A comparable average daily intake from background beef in the United States is 25.0 µg (ingestion of 0.1135 kg of beef with an average Se content of 0.22 mg/kg).
- The net increase in daily Se intake attributable to consumption of elk #51 is thus 96.7 µg (40.9 µg from elk skeletal muscle plus 80.8 µg from elk liver less 25.0 µg from background beef that the elk is replacing).
- Based on data compiled by USDA and USFDA, the average dietary Se intake in the United States, including the contribution from background beef, is on the order of 100 µg (Schubert et al., 1987—71 µg; J. Pennington, B. Young, and D. Wilson, 1989, "Nutritional Elements in U. S. Diets: Results from the Total Diet Study, 1982–1986," *Journal of the American Dietetic Association* 89, 659–85 µg; USFDA, 1982, Compliance Program Report of Findings FY 79

Total Diet Studies—Adult, USFDA, Washington, District of Columbia—139  $\mu\text{g}$  [for young adult males only]).

- The National Academy of Sciences (NAS) is about to propose an upper bound advisory daily intake of 400  $\mu\text{g}$  (W. James, FSIS, personal communication); EPA's reference dose for a median, 70-kg person equates to a daily intake of 350  $\mu\text{g}$ ; no adverse toxic effects have ever been documented below about 1,000  $\mu\text{g}$  (Yang et al., 1989a and 1989b).
- Adding the net increased intake of 96.7  $\mu\text{g}$  Se from elk liver and muscle to the average daily intake of 100  $\mu\text{g}$  results in a total dietary Se intake of about 197  $\mu\text{g}$ , a value equal to 20% of the no-observed-adverse-effects level of 1,000  $\mu\text{g}$ ; about 49% of NAS's tentative proposed advisory intake of 400  $\mu\text{g}$ ; and only 56% of the EPA's reference dose.

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## Appendix J

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**Appendix J**  
**1999 Beef Study Post-Mortem Laboratory Results**

Steer ID	Sample Date	Post-Mortem Samples—Selenium Concentrations				Post-Mortem Samples—Cadmium Concentrations	
		Skeletal Muscle (wet weight) mg/kg	Liver (wet weight) mg/kg	Kidney (wet weight) mg/kg	Heart (wet weight) mg/kg	Liver (wet weight) mg/kg	Kidney (wet weight) mg/kg
<b>Method Detection Limit</b>		0.0050	0.0050	0.0050	0.0050	0.20	0.20
1	2/9/00	0.52	0.70	1.2	0.44		
2	2/23/00	1.2	0.85	2.2	0.67	0.28	0.92
3	2/23/00	1.3	0.83	1.6	0.67	0.31	0.71
4	2/22/00	0.61	0.83	1.8	0.56	0.48	0.80
5	2/7/00	0.58	0.60	1.5	0.47		
6	2/22/00	0.92	0.91	1.7	0.58	0.56	1.3
7	2/7/00	0.84	0.84	1.6	0.55		
8	2/7/00	0.55	0.63	1.3	0.45		
9	2/23/00	0.54	0.82	1.6	0.59	0.33	0.55
10	2/9/00	0.92	0.54	1.8	0.49		
11	2/9/00	0.55	0.49	1.6	0.45		
12	2/9/00	0.54	0.51	1.5	0.46		
13	2/22/00	0.60	0.66	1.4	0.56	0.25	0.66
14	2/23/00	0.52	0.54	1.8	0.54	0.33	1.0
15	2/7/00	0.92	0.60	1.5	0.53		
16	2/7/00	0.11	0.44	1.1	0.24		
17	2/9/00	0.10	0.35	1.2	0.25		
18	2/23/00	0.098	0.41	1.4	0.27	0.19	0.31
19	2/22/00	0.11	0.46	0.93	0.26	0.21	0.55
20	2/22/00	0.097	0.45	1.6	0.26	0.16	0.33

Notes: Data validated by U of I.  
 Italicized values were reported as below detection limit (BDL). Values shown are laboratory instrument read-outs.  
 blank cells indicate no data reported.

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## Appendix K

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# **Skeletal Muscle Selenium Content of Treatment Steers Included in the Idaho Mining Association's Beef Study for the Southeast Idaho Selenium Project: A Worst-Case Analysis of Confined Animals on Reclaimed Mine Land**

A Final Report to the  
United States Department of Agriculture  
Food Safety Inspection Service

March 21, 2000

Reported Prepared by the Idaho Mining Association:

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## **Introduction**

One of the five member companies of the Idaho Mining Association (IMA) funding the Southeast Idaho Selenium Project (Se Project), Solutia Inc., conducted, in conjunction with the Idaho Department of Lands and the University of Idaho, a grazing study on the reclaimed Henry Mine in a montane setting in Caribou County, Idaho. The reclaimed land contains seleniferous waste shales, which support vegetation containing elevated concentrations of selenium (Se). This grazing study was commenced independently, but the study participants agreed to collect blood data at the end of the planned nine-week, reclaimed land grazing period that could be used for the Se Project to assess potential effects to ungulates grazing such lands and to humans who consume such ungulates.

Using the whole-blood sample information IMA estimated the average Se content in muscle at the time of slaughter to be 0.91 mg/kg (wet, all subsequent concentrations are also reported on a wet-weight basis), with a 95% upper confidence bound of 2.0 mg Se/kg. The estimated muscle Se content utilized a whole-blood to skeletal muscle correlation and depuration rate estimates. Schubert et al. (1987) indicated that background beef in the United States has an average skeletal muscle content of 0.22 mg Se/kg, with a 95% upper confidence limit of 0.39 mg Se/kg. Because there is much uncertainty in the estimate of 0.91 mg Se/kg, IMA purchased 15 steers that were part of the Henry Mine study and an additional five steers, from the same herd, that had never grazed reclaimed, seleniferous pasture. These 20 steers were transported to the University of Idaho (UI) for a depuration study to determine a better estimate of Se depuration rate and Se concentrations in various edible tissues at slaughter.

The Se Project study was initiated on October 1, 1999 with an approximate duration of 120 days. During this study, the animals were fed a finish ration that mimicked a commercial feedlot situation. The data collection phase of the depuration study has concluded. The steers were slaughtered at a rate of five per day on the 7<sup>th</sup>, 9<sup>th</sup>, 22<sup>nd</sup>, and 23<sup>rd</sup> of February. Samples of edible tissues—skeletal muscle, heart, liver, and kidney—were taken at the time of slaughter and submitted to the UI's Animal Sciences Laboratory for analysis.

Except for what was retained for analysis, all soft organs have been disposed. The carcasses were cut into primals, vacuum packed, and stored under chain of custody in a locker plant in Moscow, Idaho. All beef is clearly marked as to which animal it came from. Provided the United States Department of Agriculture's (USDA's) Food Safety and Inspection Service (FSIS) certifies the beef for human consumption after inspection and review of this muscle Se residue data, the IMA will donate the beef to the Idaho Food Bank. Upon FSIS's certification, another of the IMA member companies, J.R. Simplot Company (Simplot), will transport the vacuum-packed primals via refrigerated truck to a Simplot refrigerated storage facility in Boise, Idaho. There the primals will be further divided into consumer-sized packages before being given to the Food Bank.

Because the five control steers were not exposed to seleniferous forage there is no concern from a Se-residue perspective. As a result, they should only be subjected to standard FSIS inspection procedures. IMA believes that their meat could be handled separately from the meat derived from the fifteen treatment steers. However, for reasons of transportation and processing economy, the control beef is being handled with the treatment beef, but the control beef is labeled as such in the event that there is a problem with any of the treatment beef.

## Purpose

The purpose of this report is to provide FSIS with post-mortem Se content data in skeletal muscle from the 20 steers involved in the Se Project study and request that FSIS certify the meat as being fit for human consumption. Upon receiving certification, IMA will donate approximately 15,000 pounds of beef (approximately 11,000 pounds from the 15 treatment steers and 4,000 pounds from the five control steers) to the Idaho Food Bank. Until FSIS certifies the meat, the meat is being stored (under documented chain of custody) at a Moscow, Idaho locker plant.

In addition to the Se muscle residue data presented in this report, the depuration study generated serum, whole-blood, liver, and skeletal muscle information. However, these additional data are not presented in this report because IMA believes that they are not relevant to the decision to certify the meat from the 20 study steers. These additional data will be presented in IMA's 1999 Interim Investigation Data Report, which is currently in preparation. The additional data will be particularly relevant to assist land use managers in determining long-term grazing management decisions.

## Data

The twenty steers included in the Se Project depuration study were identified by number, 1 to 20. The steers were associated with each treatment as follows:

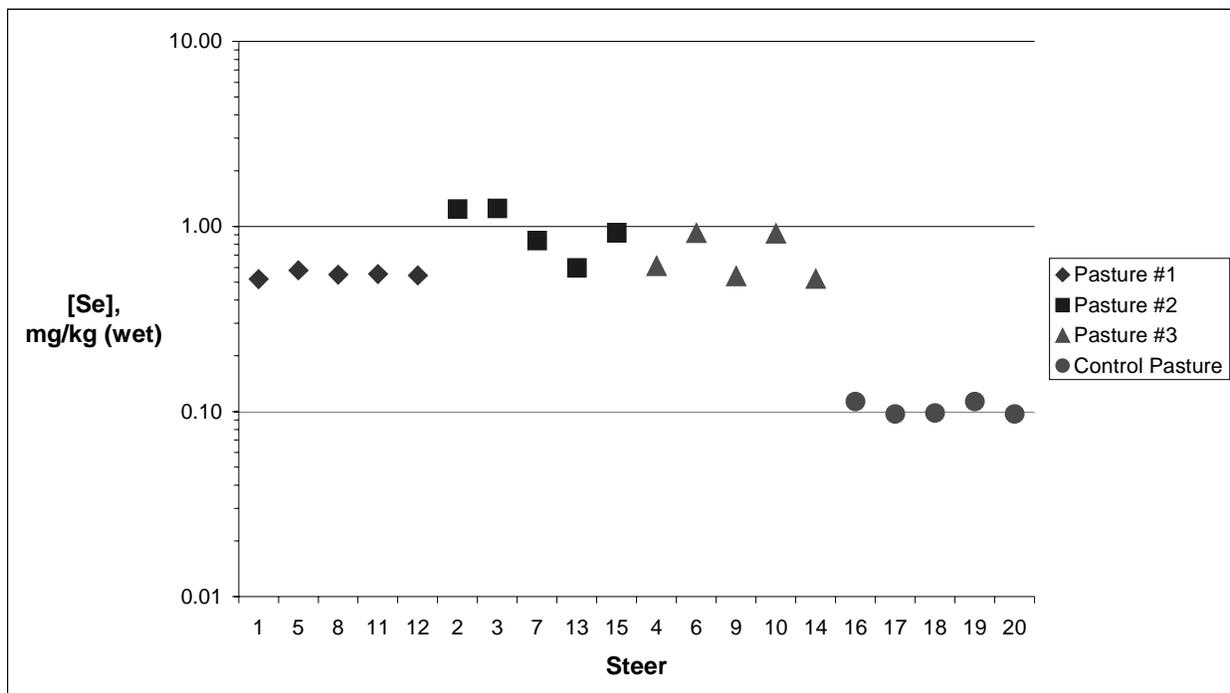
- Henry Mine Pasture #1 (nine weeks of confinement to seleniferous pasture)—steers 1, 5, 8, 11, and 12;
- Henry Mine Pasture #2 (nine weeks of confinement to seleniferous pasture)—steers 2, 3, 7, 13, and 15;

- Henry Mine Pasture #3 (nine weeks of confinement to seleniferous pasture)—steers 4, 6, 9, 10, and 14; and,
- Control (no exposure to seleniferous pasture)—steers 16 through 20.

The following table presents results of skeletal muscle laboratory analyse.

Post-Mortem Selenium Content		
<i>Steer</i>	<i>Treatment</i>	<i>mg/kg (wet)</i>
1	Pasture #1	0.519
5	Pasture #1	0.580
8	Pasture #1	0.549
11	Pasture #1	0.554
12	Pasture #1	0.543
2	Pasture #2	1.240
3	Pasture #2	1.250
7	Pasture #2	0.837
13	Pasture #2	0.597
15	Pasture #2	0.923
4	Pasture #3	0.611
6	Pasture #3	0.922
9	Pasture #3	0.539
10	Pasture #3	0.916
14	Pasture #3	0.521
16	Control	0.113
17	Control	0.097
18	Control	0.098
19	Control	0.113
20	Control	0.097

A graphical plot of these data is provided in the following figure.



## Visual Assessment

The tabulated and graphed data above are well within the range of 0.02 mg Se/kg to 3.0 mg Se/kg for beef cattle skeletal muscle reported by Ihnat (1989). The low end of the range reported by Ihnat comes from cattle raised in Ontario on a Se-deficient diet of 0.030 mg Se/kg for 330 days (Hidiroglou et al., 1985). The high end of the Ihnat's range comes from cattle raised in South Dakota on a naturally seleniferous diet of 7.6 mg Se/kg for 720 days (Moxon et al., 1944).

The diet of the South Dakota steers was comparable to that of the Se Project treatment steers. The reason that treatment steer muscle Se concentrations are substantially lower than the South Dakota study cattle is probably two-fold. First, the Se Project steers were grazed on the seleniferous pasture for a significantly shorter time period (9 weeks v. 108 weeks). Second, at the time of slaughter, the Se Project steers had undergone a minimum of 22 weeks of Se depuration. The depuration period was selected to mimic typical feedlot conditions.

The muscle Se content from the five control steers is less than 0.50 mg Se/kg. This is within the range that is generally considered non-toxic from the perspective of animal health (Puls, 1994). The tabulated and graphed data above indicate that the Se content of the various animals within each of the three treatments is higher than the control group. In addition, the graphical display shows that the Se content variance in the steers from Pastures #2 and #3 is greater than the variance observed for Pasture #1 and Control steers.

## Statistical Assessment

The average Se concentration in skeletal muscle for the 15 treatment steers is 0.74 mg Se/kg, with an upper 95% confidence bound of 0.86 mg Se/kg. These values are well below predicted concentrations of 0.91 and 2.0 mg Se/kg, respectively. However, the study results are above the background beef values of 0.22 and 0.39 mg Se/kg, respectively (Schubert et al, 1987).

The differences in the variances between the four treatment groups prevented an analysis of variance (ANOVA) to determine whether there are significant differences between the averages of the log-transformed treatment and control values. However, a  $t'$  test can be used to determine if there are significant differences. To account for the lognormal nature of environmental concentration data, the  $t'$  tests were conducted on the natural logarithms of the concentrations. The natural logarithm are presented in the following table.

Ln[Se]			
Pasture #1	Pasture #2	Pasture #3	Control
-0.656	0.215	-0.493	-2.180
-0.545	0.223	-0.081	-2.333
-0.600	-0.178	-0.618	-2.323
-0.591	-0.516	-0.088	-2.180
-0.611	-0.080	-0.652	-2.333

The critical values of  $t'$  tabulated below are experiment-wise values at an experiment-wise error rate of 0.050. These values were calculated using twelve comparisons in the overall experiment—six F tests (to evaluate homogeneity of variances) and six  $t'$  tests.

The table below indicates that there is a statistical difference between all groups; i.e., each group is distinctly different.

Means of ln-Transformed [Se]	t'	t' <sub>critical</sub>	Notes
Pasture #1 vs. Pasture #2	19.24	5.85	2-sided test at an $\alpha_{\text{experimental}}$ of 0.050
Pasture #1 vs. Pasture #3	8.40	5.85	2-sided test at an $\alpha_{\text{experimental}}$ of 0.050
Pasture #1 vs. Control	104.96	4.82	1-sided test at an $\alpha_{\text{experimental}}$ of 0.050
Pasture #2 vs. Pasture #3	8.56	5.85	2-sided test at an $\alpha_{\text{experimental}}$ of 0.050
Pasture #2 vs. Control	77.45	4.82	1-sided test at an $\alpha_{\text{experimental}}$ of 0.050
Pasture #3 vs. Control	71.74	4.82	1-sided test at an $\alpha_{\text{experimental}}$ of 0.050

## Human Health Assessment

In humans, chronic exposure to high doses of Se ( $> 1,000 \mu\text{g}/\text{d}$ ) can result in dermatological effects, including thickened and brittle fingernails, loss of hair, or itchy skin. These symptoms are reversible, and adults are more sensitive than children (Yang et al., 1989a; 1989b).

Neither the FSIS nor the United States Food and Drug Administration (USFDA) have Se residue standards for food at this time. The USFDA has used a 1 mg/kg decision criterion in the recent past for an event of Se poisoning of swine in California (SOURCE). Australia has promulgated Se standards for beef:

- 2 mg Se/kg for edible offal; and,
- 1 mg/kg for skeletal muscle.

All of the Se Project steers are below Australia's 2 mg Se/kg standard for offal. Two steers, No. 2 and No. 3, had skeletal muscle Se concentrations of 1.24 and 1.25 mg Se/kg, respectively. These values are greater than then both the Australian standard of 1 mg Se/kg for muscle and USFDA's recent decision criterion that was applied to the California swine. However, the average of the 15 fifteen treatment steers, 0.74 mg Se/kg (with an upper 95% confidence bound of 0.86 mg Se/kg), is well below the 1 mg Se/kg standard/criterion.

A simple conservative bounding assessment of risk, presented below, is used to demonstrate that even steer No. 3, with a 1.25 mg Se/kg content, is safe to consume on a chronic-consumption basis:

- Assume that one eats 4 oz, or 0.1135 kg, of beef per day. This is approximately twice the national average (U.S. Environmental Protection Agency [EPA], 1996).
- Assume a Se content of 1.25 mg Se/kg, which was the maximum muscle concentration in any of the study steers.
- These assumptions equate to a maximum daily Se intake, from treatment beef, of 0.142 mg, or 142  $\mu\text{g}$ .
- A comparable average daily intake from background beef in the United States is 25.0  $\mu\text{g}$  (ingestion of 0.1135 kg of beef with an average content of 0.22 mg Se/kg).
- The maximum net increase in daily Se intake equals 117  $\mu\text{g}$ .
- Based on data compiled by USDA and USFDA, the average dietary Se intake in the United States, including the contribution from background beef, is on the order of 100  $\mu\text{g}$ . Schubert et al.(1987) reported a Se intake of 71  $\mu\text{g}$ . Pennington et al. (1989) reported a daily intake of 85  $\mu\text{g}$ . A USFDA (1982) study of young adult males reported a daily Se intake of 139  $\mu\text{g}$ .

- The National Academy of Sciences (NAS) is prepared to propose an upper bound advisory daily intake of 400  $\mu\text{g}$  (W. James, personal communication). Yang et al. (1989a and 1989b) report that no adverse toxic effects have ever been documented below about 1,000  $\mu\text{g}$ . Both of these values are chronic intakes.
- Adding the maximum net increased intake of 117  $\mu\text{g}$  to the average daily intake of 100  $\mu\text{g}$  results in a total dietary Se intake of about 217  $\mu\text{g}$ , a value only 22% of the no-observed-adverse-effects level of 1,000  $\mu\text{g}$ , and only 54% of the NAS's tentative proposed advisory intake of 400  $\mu\text{g}$ .

The bounding assessment of risk uses chronic intake estimates and health advisory levels based on chronic intakes. The IMA will be donating the beef to the Idaho Food Bank, which will then distribute the beef to needy recipients. Because the beef will be widely distributed, no recipient will receive enough beef to constitute a chronic exposure. Additionally, the assessment is based on the maximum Se content observed in the Se Project study steers. It is also assumed that meat from the 20 study steers, 15 treatment and five control, will, by the time the beef is distributed, be randomly mixed by the processing, storage, transportation, and distribution activities. (Please note that the meat is currently labeled by steer and maintained under chain of custody during FSIS's evaluation and inspection.) Consequently, a recipient of the beef will probably receive packages originating from more than one steer.

Therefore, the average of all 20 steers is a more appropriate exposure concentration than is the maximum value observed. The 1.25 mg Se/kg concentration used in the bounding assessment should be replaced by the average for all 20 steers, which is 0.58 mg/kg. This results in the reduction of the estimated total daily intake to 154  $\mu\text{g}$ .

## Summary

IMA requests that FSIS certify the 20 Se Project steers as safe for human consumption from the perspective of Se content in skeletal muscle. Upon receipt of the certification the IMA will donate the beef to the Idaho Food Bank. In this report IMA has shown with the post-mortem data that:

- The data are well within the range of beef muscle Se concentrations reported from the United States (i.e., beef raised on naturally seleniferous range in areas of such states as South Dakota, North Dakota, and Wyoming have concentrations as high or higher and such concentrations have never been held up as a consumption hazard);
- All but two of our fifteen treatment steers are well below the Australian standard of 1 mg Se/kg, a benchmark USFDA has used in the recent past as a decision criterion involving swine;
- The average Se concentration of the 15 treatment steers and the corresponding 95% upper confidence limit are well below the standard/criterion of 1 mg/kg;
- Chronic intake of the treatment steer beef is virtually impossible; and,
- Even if a chronic intake were possible, it would be highly unlikely that the NAS's tentative proposed advisory intake of 400  $\mu\text{g}$  would be exceeded, virtually impossible to exceed the daily chronic toxic effects threshold intake of 1,000  $\mu\text{g}$  reported by Yang et al. (1989a and 1989b).

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## Appendix L

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**ANNUAL REPORT**

**Genetic Variation Among Cutthroat Trout (*Oncorhynchus clarki*) in the  
Blackfoot River, Idaho**

**for**

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**June 2, 2000**

## Specific Objectives

1. To determine the taxonomic status of cutthroat trout samples collected from the Blackfoot River and Henry's Lake Hatchery in 1999.
2. To determine if cutthroat collected from Henry's Lake, ID, being used in selenium diet experiments, are genetically different from those collected from the Blackfoot River.
3. To determine if cutthroat trout from the Blackfoot River watershed lack genetic variation as compared to cutthroat populations from adjacent areas.

## Introduction

The management and conservation of cutthroat trout have become priorities for several state and federal agencies due to the decline of Yellowstone cutthroat trout populations throughout their historic native range (Thurow et al. 1988). In August 1998, several conservation groups petitioned the U. S. Fish and Wildlife Service to list the Yellowstone cutthroat trout as a threatened species under the Endangered Species Act (ESA). Currently, the Idaho Department of Fish and Game recognizes all cutthroat trout as a "species of special concern" (Thurow et al. 1988). Several environmental and anthropogenic factors may be contributing to the decline of these fish. The University of Idaho's (U of I's) Center for Salmonid and Freshwater Species at Risk is collaborating in a multidisciplinary effort to genetically characterize the cutthroat trout subspecies throughout the state of Idaho (Powell 2000).

The purpose of this study is to employ molecular genetic techniques to directly assess genetic variation within and among cutthroat trout in the Blackfoot River and to assess their genetic similarity to experimental fish collected from Henry's Lake and Willow Creek, Idaho. The advantage of molecular-based techniques lies in the ability to quantitatively assess genotypes rather than phenotypes (i.e., genetic composition rather than appearance).

Isozyme analysis has been used to examine the relatedness of individuals among and between populations of cutthroat trout. (An isozyme is one of two or more molecular forms of the same enzyme.) This technique as with all others, has its limitations. The level of variation and rate of mutation in isozymes may fall short of being able to detect a minimal loss of genetic variation within this population. Moreover, isozyme analyses typically require destructive sampling of tissue and organs. This aspect of isozyme analysis limits its usefulness in this situation (see Mitton 1997 for a review).

We instead employed the use of restriction fragment length polymorphism (RFLP) analysis on a polymerase chain reaction (PCR) amplified region of mitochondrial DNA (mtDNA). This technique is relatively simple and non-destructive. Mitochondrial DNA is inherited in a clonal (non-recombinatory) fashion from the female. This allows for direct assessment of maternal genealogy and dispersal. Mitochondrial DNA lacks similar enzymes that edit mistakes made during DNA replication in the nuclear genome.

Thus, the mutation rate in mtDNA can be much higher (up to 10-fold higher) than mutation rates observed in nuclear DNA (nDNA) sequences.

These attributes—simplicity, non-destructive sampling, and high mutation rate—make mtDNA RFLP analysis an attractive choice for investigating genetic variation within and among populations of cutthroat trout. Most importantly for this study, since mtDNA is only inherited from the female and is non-recombinatory, it effectively reduces the population size to  $\frac{1}{4}$ . Thus, mtDNA is very sensitive to any potential loss of genetic variation the assessment of which is the experimental objective (see Avise 1994 for a review).

We also chose to examine two nuclear gene loci within the samples to add a second independent data set and compliment estimates of genetic variation based solely upon diversity within mitochondrial lineages. The two nuclear gene regions examined were Ikaros gene, IK, and recombination activation gene, RAG3'. These gene regions were examined using RFLP analysis similar to the analysis of the NADH dehydrogenase 2 (ND2) mitochondrial gene region described above.

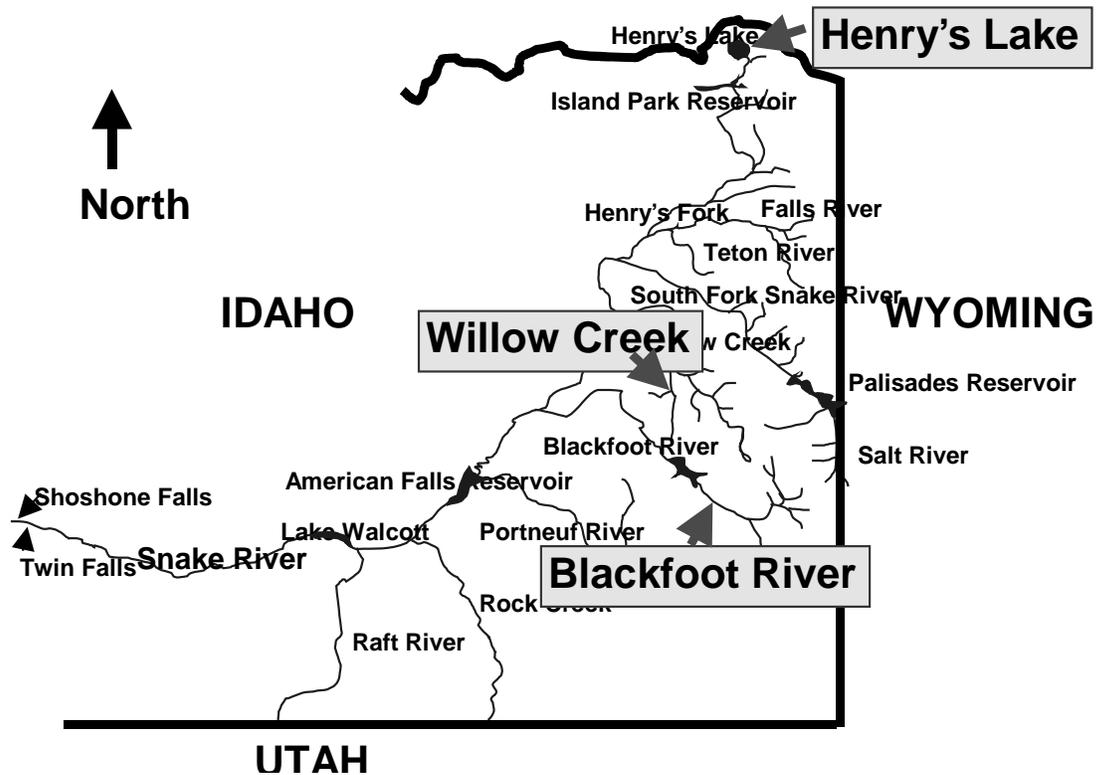
## Methods

### Biochemistry.

Fin samples were collected from cutthroat trout and stored in 70% ethanol or preservation/lysis buffer until DNA was extracted using methods modified from Sambrook et al. (1989) and Dowling et al. (1990). Figure 1 shows the approximate locations of sample collection. Total genomic DNA was isolated from each sample and amplified using the PCR and nucleotide primers specific for the ND2 gene region of the mitochondrial genome (ND2, #562 5'TAA GCT ATC GGG CCC ATA CC<sup>3'</sup> and #461 5'GGC TCA GGC ACC AAA TAC TAA<sup>3'</sup>) as well as primers for the Ikaros (IK-F1 and IK-R1) and RAG3' (RAG3'-F1 and RAG3'-R1) nuclear gene regions (Baker and Moran unpublished).

Amplification products were digested with specific restriction enzymes (*Rsa* I, *Hinf* I and *Dde* I respectively) to produce diagnostic banding patterns. The resulting DNA fragments were separated by electrophoresis using 3% agarose/TAE gels. Vertical 6% polyacrylamide/TBE gels were also used to separate small fragments and questionable co-migrating fragments. Restriction fragment length polymorphisms observed among samples were given alphabetical designations as haplotypes (for mtDNA) or alleles (for nDNA). The size of each DNA fragment from each gene region was estimated by comparison to a size standard, pUC-19 marker (Bio-Synthesis).

**Figure 1.** Map of the Snake River above Shoshone Falls detailing sample collection areas.



The resulting haplotypes were compared to two other populations of cutthroat trout (*Oncorhynchus clarki bouvieri*) and a different subspecies, westslope cutthroat trout (*Oncorhynchus clarki lewsi*) to add geographic and phylogenetic perspective to the analysis. The resulting genotypes were compared for significant differences among populations.

#### Population and Statistical Analyses.

An estimate of the number of nucleotide substitutions per site ( $p$ ) for each RFLP was calculated via the Nei (1987) method using REAP 4.0 (McElroy et al. 1991). These estimates were then used to generate a matrix comparing  $p$  values (distance) between all pairs of identified haplotypes. Previous work in our laboratory has examined a more extensive set of RFLP analyses within cutthroat trout. The results are summarized here as they relate to genetic distance among the different subspecies. The KITSCH program in PHYLIP 3.5 (Felsenstein 1993), which assumes independence and equal rates of divergence, was used to generate a distance dendrogram using the least-squares method of Fitch and Margoliash (1967) to illustrate the estimated evolutionary relationships and distance among the identified haplotypes. The extent of geographic heterogeneity among population frequency distributions was examined using a Monte Carlo simulation of a chi-square analysis with 1,000 iterations (MONTE program in REAP ver. 4.0).

Nucleotide diversity and divergence among and within populations was estimated using Nei (1987) equations 10.19, 10.7, 10.20, and 10.21 in the DA program of REAP ver. 4.0.

Thus, the genetic relationship of Blackfoot River cutthroat trout to a nearby population and an experimental population was ascertained along with the extent of genetic variation as it relates to mtDNA. The level of nuclear genetic variation in the Blackfoot population was compared to that of the other populations to assess whether or not a loss of variation has occurred.

## Results

### Taxonomic Status.

Review of literature on life history and geographic distribution among cutthroat trout subspecies indicate the cutthroat trout within the Blackfoot River are *Oncorhynchus clarki bouvieri* or Yellowstone cutthroat trout (Behnke 1992). This is confirmed by mtDNA RFLP analysis which can distinguish among these subspecies (see Williams et al. 1998 for a phylogeny based upon mitochondrial RFLP variation).

### Mitochondrial Variation.

Table 1 shows the comparison of nDNA and mtDNA variation present among the Blackfoot population as compared to the Henry's Lake Hatchery and Willow Creek populations. Three mitochondrial lineages are present in the Blackfoot population with haplotype B being the most prevalent at 83.3%. Willow Creek samples were fixed for this mitochondrial lineage. Conversely, the Henry's Lake population, though also comprised of three mitochondrial lineages, contained significantly different frequencies of each lineage.

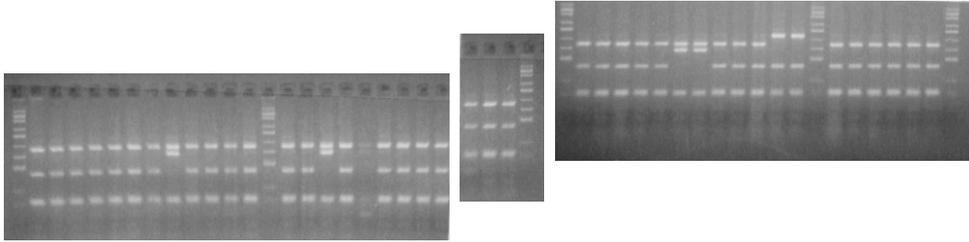
In the Henry's Lake Hatchery population, D is the most prevalent haplotype at 77.8% as opposed to B, which only has a frequency of 15.6%. Mitochondrial haplotype pattern A, which is diagnostic for rainbow trout, was absent in all three populations. Thus, evidence of introgressive hybridization with rainbow trout at the mitochondrial locus was not observed.

**Table 1.** Genetic variation among three Snake River Basin populations of cutthroat trout at one mitochondrial and two nuclear loci.

Population	N	mtDNA				nDNA					
		ND2 gene / <i>Rsa</i> I				Ikaros gene / <i>Hinf</i> I			RAG3' gene / <i>Dde</i> I		
		A	B	C	D	A	B	C	A	B	C
Blackfoot River	42	0.000	0.833	0.047	0.120	0.000	1.000	0.000	0.000	0.427	0.573
Henry's Lake	45	0.000	0.156	0.067	0.778	0.000	1.000	0.000	0.000	0.637	0.363
Willow Creek	48	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.438	0.563

Figure 2 shows the ND2 RFLPs for the Blackfoot population.

**Figure 2.** Archived gels of Blackfoot samples (ND2 gene PCR amplified and cut with *Rsa* I) showing mitochondrial variation.



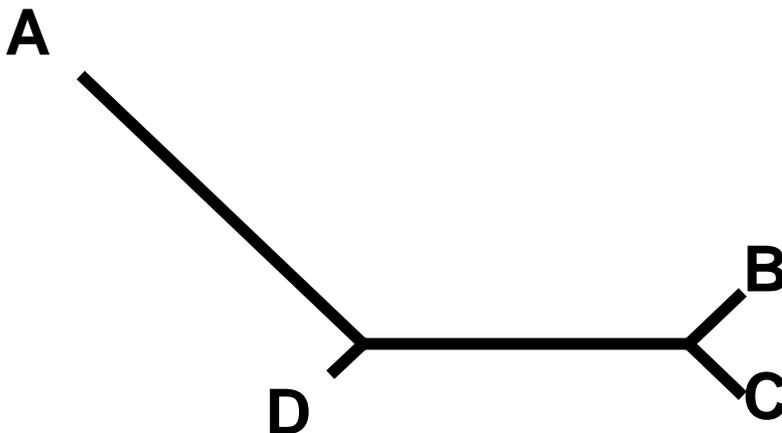
### Nuclear Variation.

The Ikaros gene region RFLPs showed no variation and was fixed for the B allele among all three populations. However, variation has been observed at this locus in other cutthroat populations in the Snake River (Campbell and Powell unpublished). The RAG3' locus was variable in all three populations. Allele frequency differences between the Blackfoot River and Willow Creek were non-significant, whereas allele frequency differences between these two populations and the Henry's Lake Hatchery population were significant. Allele frequencies at the RAG 3' locus vary considerably among other cutthroat populations studied thus far with the B allele ranging from 11.3 to 92.7% in frequency (Campbell and Powell unpublished). Alleles diagnostic for rainbow trout were not observed at either nuclear locus.

### Genetic Distance.

The estimated genetic divergence between haplotypes (shown in Figure 3 from Williams et al. 2000) indicates B, C, and D are closely related (sequence divergence is <0.5%) and cluster separately from A, which is a rainbow trout haplotype (sequence divergence is >2%).

**Figure 3.** Yellowstone cutthroat mtDNA phylogeny inferred using the Fitch-Margoliash pair-wise distance method and a neighbor-joining tree algorithm.



All three haplotypes are found among populations of Yellowstone cutthroat trout in the upper Snake River Basin. The presence of the A haplotype, as previously stated, would indicate rainbow trout introgression but was not observed within the three populations in this study.

### **Discussion and Recommendations**

Mitochondrial genetic analysis confirmed the samples collected from the Blackfoot River and those used for experimental diet studies containing selenium (both Blackfoot and Henry's Lake samples) are Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*). Comparison to westslope cutthroat trout showed fixed mitochondrial differences among haplotypes (data not shown, see Williams et al. 1998). Moreover, no evidence of rainbow trout introgression was observed in any samples at any of the three loci examined. Concerns over whether or not the Henry's Lake cutthroat trout used in the experimental diet studies are introgressed with rainbow trout and thus may somehow react differently to selenium exposure are reduced. However, the probability of some very low level of introgression undetected in this sample set still exists primarily as a consequence of known hybridization and a history of stocking of rainbow trout in Henry's Lake.

Fish collected from a Snake River tributary (Willow Creek) in geographic proximity to the Blackfoot River did not contain significantly different allele frequencies at two nuclear loci. This information fails to support any hypothesis that Yellowstone cutthroat trout in the Blackfoot River have less genetic variation than conspecifics in other streams, which are putatively unaffected by increased levels of selenium. In comparison to several additional populations thus far examined (15), Blackfoot River cutthroat trout have comparable variation to any of the other locations (these data are being prepared for publication in an AFS symposium proceedings by Williams, Powell, and Campbell). Interestingly, Willow Creek samples were also fixed for a single mitochondrial lineage as opposed to the three lineages found in the Blackfoot and Henry's Lake samples. Even though the Ikaros locus was fixed among the three populations examined, it is variable in other populations used in the ongoing genetic characterization of cutthroat trout by the U of I.

Some caution should be exercised not to over generalize these results based upon two loci. Several nuclear loci with significant variability such as microsatellites may be required to detect any lack of genetic variation within the Blackfoot River population as compared to other Yellowstone cutthroat populations. This is supported from additional observations that the Yellowstone cutthroat trout subspecies is presumed to be moderately invariant as compared to some other cutthroat subspecies such as westslope cutthroat trout (Williams personal communication). How a lack of genetic variation may ultimately relate to Yellowstone cutthroat fitness or a lack thereof is also debatable. A lack of genetic variation can be the result of several natural phenomena completely unassociated with putative selenium effects. Until a selenium effect can be demonstrated in the population, any detected "loss of genetic variability" should be considered coincidental, not correlative.

### Recommendations.

Genetically, there is no evidence to support a hypothesis that Henry's Lake Hatchery cutthroat would not serve as an appropriate surrogate to test for selenium effects in Yellowstone cutthroat trout. Yellowstone cutthroat trout are not that different from each other. However, obtaining and testing fish from Willow Creek would alleviate some of those concerns, since they appear, in so far as they have been tested, to be very similar to Blackfoot River cutthroat. These fish are also from a nearby location outside the area of elevated selenium exposure.

Obtaining fish from Willow Creek could also reduce variability in the feeding studies in another way. The Henry's Lake fish have been propagated in a hatchery for nearly 30 years and are easier to captively raise. Conversely, wild fish and wild cutthroat in particular are difficult to raise in captivity. Thus, the effects of captive propagation on wild fish may be difficult to completely tease away from effects associated with the experimental diets unless of course you are also raising similar, wild cutthroat from a nearby location.

### Summary.

With respect to the three specific objectives of this study, the results are summarized as follows:

1. To determine the taxonomic status of cutthroat trout samples collected from the Blackfoot River and Henry's Lake Hatchery in 1999.

The cutthroat trout from both the Blackfoot River and Henry's Lake are Yellowstone cutthroat trout, *Oncorhynchus clarki bouvieri*. There is no evidence of hybridization with rainbow trout in either population. As such, both populations could be subject to management under the ESA in the future.

2. To determine if cutthroat collected from Henry's Lake, ID, being used in selenium diet experiments, are genetically different from those collected from the Blackfoot River.

On the basis of both mtDNA and nDNA analyses, the population of Yellowstone cutthroat trout in the Blackfoot River is different from the population of Yellowstone cutthroat trout in Henry's Lake. Because they are the same subspecies, the Henry's Lake fish are likely good controls and surrogates for the Blackfoot River fish. However, an alternative control population, from Willow Creek, is much more closely related to the Blackfoot River population, showing no evidence of difference on the basis of nDNA analysis. Another advantage of using Willow Creek fish in future

studies as controls is that they, like the Blackfoot River fish, are wild, unlike the hatchery raised fish from Henry's Lake.

3. To determine if cutthroat trout from the Blackfoot River watershed lack genetic variation as compared to cutthroat populations from adjacent areas.

There is no evidence to indicate that the population of Yellowstone cutthroat trout in the Blackfoot River have less genetic diversity than what is observed in Yellowstone cutthroat trout populations that do not dwell in seleniferous habitats. Thus, there is no evidence that empirical investigations of the Blackfoot River population of Yellowstone cutthroat trout will be affected by survivorship bias.

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