

# **SELENIUM BIOACCUMULATION IN SELECTED STREAM AND LAKE FISHES OF WEST VIRGINIA**

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## **ABSTRACT**

Selenium toxicity poses a threat to organisms inhabiting aquatic ecosystems influenced by excessive seleniferous inputs. It is also an essential nutrient in living things and provides antioxidant properties to tissues. Variability in the literature regarding a suggested tissue-based criterion for freshwater fishes and subsequent protective water quality concentrations indicate the complexity of the bioaccumulative processes surrounding selenium. While sublethal considerations of selenium toxicity must be thoroughly examined in experimental settings, the manifestation of deleterious effects serves an equally important role in the identification of problematic levels and establishment of appropriate thresholds. Thus, a study was designed to determine the bioaccumulation rates of selenium in the fishes of West Virginia's streams and lakes, and identify potential toxic effects among influenced populations. Focusing on potentially vulnerable populations, the research will seek to clarify differences in the biological uptake of selenium in contrasting aquatic environments and elucidate inter- and intraspecific relationships of variable selenium tissue concentrations through basic interpolation.

## **INTRODUCTION**

In recent years, the toxicity of selenium in aquatic environs has drawn much attention. This has been largely due to heightened awareness of the anthropogenic sources of selenium and the implications of uncontrolled contamination (Quinn and Vorster, 1998; Lemly, 1999; Holm *et al.*, 2003). Of particular scientific focus have been the mechanisms through which the introduction of selenium into aquatic systems occurs, the processes by which it is assimilated and transferred throughout the food web—bioaccumulation, the thresholds of its toxicity to certain organisms, and the remediation of selenium-contaminated environments. Although extensively researched for the past two decades, the ultimate role and fate of selenium in biological systems is still quite contentious and highly variable (Reash *et al.*, 1999; Brix *et al.*, 2000).

Selenium is a non-metallic element that has a normal low-level presence in surface waters (Lemly, 1997; Irwin *et al.*, 1997). Sources of selenium in aquatic systems include runoff from soils rich in marine sedimentary deposits, leaching from seleniferous soils via agricultural irrigation, the combustion of fossil fuels (including waste disposal), various mining practices that expose rock strata, and petroleum contamination (Quinn and Vorster, 1998; Mauk and Brown, 1999; Swift, 2002). In a given aquatic environment, selenium may occur in many different forms; however, it has been suggested that the valence state of biologically available selenium (either  $\text{Se}^{+4}$ , selenite,  $\text{Se}^{+6}$ , selenate, or  $\text{Se}^0$ , elemental selenium) factors greatly into its toxicity (Chapman, 2000). Furthermore, the presence of other potentially antagonistic constituents (e.g. Pb, Cu, As, and sulfate) may reduce the toxic effects of selenium (Lohner *et al.*, 2001). Once selenium contamination has occurred, there are contrasting views as to the potential for successful environmental remediation. Some studies indicate that bacteria may be useful in selenium transformations (Stolz *et al.*, 1999; Dungan *et al.*, 2003); however, other authors predict more dire (long-term) consequences to excessive selenium inputs (Lemly, 1999).

In a biological sense, selenium serves as a micronutrient essential to life functions, and occupies a relatively narrow range of acceptable concentration. In animals, both organic and inorganic forms may be utilized, and selenium is purported to prevent tissue damage incurred through oxidative processes; however, at levels not much greater than the necessary amounts, selenium can be poisonous (Irwin *et al.*, 1997; Burk, 2002). Selenium has been documented to primarily enter organisms through dietary pathways, which predisposes certain trophic levels to the effects of biomagnification, or the amplification of a toxicant in consumers above the amount contained in their food (Lemly, 1997; Stolz *et al.*, 1999). However, other routes of exposure certainly exist, especially in standing water where contaminated sediments may persist (Holm *et al.*, 2003). Yet, fishes may be more susceptible than other organisms through certain vulnerable life history stages and a reliance on food items like some macroinvertebrates shown not to be affected by exposure to selenium (Swift, 2002).

At the molecular level, the evidence of selenium toxicity stems from its substitution for sulfur in thiol-containing proteins. More specifically, selenium may substitute for sulfur in the amino acids cysteine and methionine, changing their structural confirmation and altering their function when incorporated into proteins. In life stages where organisms are more dependent on proteins (e.g. yolk-sac larvae), these selenoamino acids may be particularly detrimental (Stolz *et al.*, 1999; Chapman, 2000). Consequently, early embryological development as well as larval organisms may be disproportionately influenced by selenium exposure, which may result in gross (teratogenic) deformities in extreme cases (Lemly, 1997). As organisms mature, the impacts of excess selenium exposure may manifest themselves in several ways. Hematologically, the components of the blood may be significantly altered (Lohner *et al.*, 2001), and/or certain tissues affected (e.g. the incurrence of hepatopancreatic and gonadal edema). Lemly (1997) details an extensive list of symptoms of chronic selenium poisoning in fish, which include respiratory, renal, and reproductive pathways. As a result, populations may be

compromised through reproductive failures, ranging from impacts on gametes to the transfer of selenium from females to their eggs during vitellogenesis (Holm *et al.*, 2003). Irrespective of the acceptance of the detrimental implications of selenium toxicity, there continues to be much contention surrounding the levels of exposure required for the manifestation of toxic effects in populations (Hamilton and Lemly, 1999; Brix *et al.*, 2000; Adams *et al.*, 2000). Consequently, there has been much uncertainty in the literature regarding the realized influence on bioaccumulation.

A range of bioaccumulation factors have been suggested in the literature, and there are two primary sources of this variability: the experimental derivation of different toxicity thresholds between and among species, and contrasting experimental settings (e.g. lotic versus lentic, or laboratory designs). In fact, some authors suggest the development of site-specific selenium bioaccumulation factors as the only reliable way to reconcile variability observed in fish tissue concentrations versus measured selenium values in the water column and/or sediment (Adams *et al.*, 2000). Furthermore, depending on the species studied and the bounds of subjectivity through which the realized effects of toxicity were measured, an assortment of protective concentrations for selenium exposure may result from similar experiments/observations (Mauk and Brown, 1999; Brix *et al.*, 2000; Swift, 2002; Kruse and Scarnecchia, 2002; Holm *et al.*, 2003; Saiki *et al.*, 2004).

In toxicological scenarios, however, sublethal effects must be considered and should represent the organisms most vulnerable to toxicity. USEPA (2004) suggests a chronic exposure whole-body tissue criterion based on bluegill sunfish, *Lepomis macrochirus*, as a value protective of the species most susceptible to the sublethal effects of elevated selenium concentrations. Based on the results of laboratory experimentation, it was determined that a whole-body tissue concentration of 7.91 µg/g should not be exceeded if individuals of this species were to remain asymptomatic for selenium toxicity. In the supporting study, it was hypothesized that increased selenium exposure in combination with low temperatures could produce a reduction in body condition, including lowered lipid content, in a condition termed Winter Stress Syndrome (Lemly, 1993). Yet, several authors contend that this criterion is overly protective and/or simplistic and provides evidence that in certain environs, exposure to elevated selenium concentrations to the degree that the suggested tissue criterion is exceeded in individuals of this species do not result in the symptoms of sublethal selenium toxicity, upon which the criterion was based (Reash *et al.*, 1999; Lohner *et al.*, 2001).

Therefore, due to such variability in the literature regarding a chronic exposure whole-body tissue criterion protective of the most sensitive species of freshwater fish, it was determined that a regional study designed to elucidate the factors and impacts of selenium bioaccumulation among select fish species, including bluegill sunfishes, found in the surface waters of West Virginia is warranted. This research will emphasize the correlation of observed whole-body tissue concentrations of selenium in fishes to in-stream selenium quantities in both lotic and lentic environments. Particular attention will be given to potentially more susceptible sunfishes (family Centrarchidae); however, other species found in potentially affected aquatic systems will also be researched.

## MATERIALS AND METHODS

### WATER CHEMISTRY

Site-specific bioaccumulation factors for selenium will be ascertained for select species of stream and lake fishes at 15 locations (Fig. 1). Water chemistry monitoring will occur at sites with known or suspected elevated levels of selenium. Monitoring sites were identified after researching information contained in the Total Maximum Daily Load (TMDL), Watershed Assessment, Mining and Industrial Permitting Section databases. Generally, selected stream and lake monitoring locations are downstream of surface mining/valley fill areas and fly ash disposal sites, with the exception of the uninfluenced control sites. Selenium concentrations will be monitored at these locations monthly for one year, and will be analyzed at minimum detection limits  $\leq 1 \mu\text{g/L}$ . Chemical analyses for other metals/ions will also be performed quarterly on the collected water samples, with particular focus on the possible antagonistic relationship between selenium and certain constituents (e.g., copper, mercury, lead, zinc, cadmium, arsenic, and sulfates). Stream flow will also be measured during each sampling event using a Marsh McBirney® Flo-Mate™ meter, in the units of cubic feet per second.

### FISH TISSUE

Stream fishes will be collected by backpack electroshocking; whereas, lake fishes will be collected by gill nets, hook and line, and electroshocking methods. Several species, including bluegill sunfish, green sunfish, creek chub, central stoneroller, rockbass, and white sucker, will be selected, where available, for whole-body tissue analysis for selenium, and will provide a means for interspecific bioaccumulation comparisons between and among sites. Fish collections will occur once in summer/fall and winter/early spring at each proposed location. Collected specimens will be individually labeled (tagged), double bagged, and iced to 4°C in transit to storage; specimens will be stored at <0°C in preparation for laboratory tissue analysis. Tissue analysis to determine whole-body concentrations of (dry weight) selenium ( $\mu\text{g/g}$ , or micrograms of selenium per gram of fish tissue), will be prescribed for all specimens in consistency with EPA's suggested whole-body chronic exposure tissue criterion of 7.91  $\mu\text{g/g}$  (dry weight selenium) for bluegill sunfish (USEPA, 2004). Specifically, EPA Laboratory Analysis Method 200.3 will be followed for the derivation of whole-body selenium concentrations in fish tissue (USEPA, 1991). Individual fish will be analyzed, representing as many analyses as five fish per species per collection event. Intraspecific compositing may be necessary if only small fishes are collected (<10 cm) in order to achieve a desired minimum sample weight of 5 g. A thorough inspection of all fishes for anomalies/disease will be completed, with particular attention given to overall fitness and the symptoms of Winter Stress Syndrome (Lemly, 1993). Other species may be preserved (frozen as whole specimens) during the collection efforts for potential future analyses.

### BIOACCUMULATION FACTORS

Upon collection, analysis and performance of appropriate final quality assurance/quality control measures, the data will be used in a series of bioaccumulation rate calculations following EPA protocols. Separate calculations will be used to derive the bioaccumulation rates in fish collected from streams and lakes. Furthermore, any and all combinations of site-specific bioaccumulation factors will be calculated and analyzed for trends. Generally, the following equation will be used to calculate bioaccumulation factors for the collected fish:

$$\text{BAF} = \text{Tissue Concentration} / \text{Water Column Concentration}$$

### **PROJECT STATUS**

The study of the bioaccumulation and potential toxic effects of selenium in selected stream and lake fishes of West Virginia is underway. Currently, the research is in the data collection phase, with both initial water chemistry collections and comparative fish tissue surveys accomplished. However, due to the nature of the study which involves the interpolation of tissue concentrations between and among species, and the overall correlation of these values to the water chemistry vectors of selenium exposure, preliminary results are unavailable at this time.

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Figure 1. Streams and lakes selected for selenium monitoring in regard to water column concentrations and bioaccumulated fish tissue.