Technical Memorandum

Technical Briefing Paper on Selenium

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Selenium

Introduction

This technical memorandum summarizes information related to exposure and risks from selenium in the environment. The purpose of this summary is to provide useful technical background information for understanding the potential ecological consequences related to the December 2008 failure of a coal ash containment dike at TVA's Kingston Fossil Plant in East Tennessee. This spill resulted in coal ash entering into two sloughs that flow into the Emory River upstream of its confluence of the Clinch River. Coal ash also was deposited above the waterline in the affected sloughs and in and along the banks of the Emory River. Recovery efforts have been ongoing by TVA to stabilize and remove ash from the waterways and land. Because ecological effects are of most concern for selenium in aquatic environments, this report focuses on environmental rather than human health risks. This briefing paper summarizes the state of the science from the technical literature and regulatory agency reports within the available time and resources for this project. It does not specifically quantify risks from selenium coal ash released from this event. This information could be used along with the site data resulting from ongoing investigations to support assessment and communication of risks.

Selenium is a metalloid that is next to sulfur on the periodic table and shares many of the same properties. It is element number 34, with a molecular weight of 78.93. It was discovered in 1817 by the Swedish chemist Jöns Berzelius, who was the first to differentiate it from tellurium and named it after Selene, the Greek goddess of the moon (McDowell 2003). Although selenium's toxicological properties were not defined until the 1930s, when scientists from the U.S. Department of Agriculture identified selenium as the toxic element in plants that causes "alkali disease" in horses and livestock, its effects were recognized as early as the 13th century when horses in Marco Polo's expedition across Asia sloughed their hoofs as a result of grazing on selenium hyperaccumulating plants (NRC 1980). Several species of desert plants (e.g., *Astragalus* spp.) hyperaccumulate selenium because of its chemical similarity to sulfur (Galeas et al. 2006). Horses, cattle, and other livestock that graze on these plants characteristically

exhibit brittle haircoats, hair loss, sloughed hooves, and at sufficiently high concentrations, sudden death. In 1957, scientists reported that selenium is an essential nutrient in animals (Mayland 1994); it is not yet clear whether it plays a similar role in plants (Galeas et al. 2006; Mayland 1994).

Environmental impacts of selenium came to the public's attention with two high-profile incidents in the 1980s. Belews Lake, North Carolina, suffered a severe fish die-off and the loss of 19 species of fish when ash from a coal-fired power plant was discharged directly into the lake. On the other side of the country, severe deformities were observed in birds at Kesterson Wildlife Refuge, California, which was contaminated with selenium from discharges of agricultural drainwater. Since then, it has become apparent that selenium can cause significant effects to fish and aquatic dependent wildlife as a result of its ability to bioaccumulate in the aquatic food web. However, it also is now known that accumulation is highly dependent upon the type of selenium at the source and the biogeochemistry of the receiving environment. This paper reviews the state-of-the-science of the potential for adverse environmental effects to occur as a result of selenium contamination associated with ash from coal-fired power plants.

Selenium in the Environment

Selenium exhibits complex geochemical behavior in the environment. The chemical form of selenium can change depending on pH, presence or absence of oxygen, and the concentration of other elements, particularly iron, manganese, and aluminum oxides.

According to Mason (1966), selenium ranks 34th in elemental abundance (following arsenic) of 92 elements occurring naturally in the earth's crust, with an average concentration of 0.05 mg/kg. Other sources rank selenium's abundance differently. Although the average concentration is consistently low, the distribution of selenium is uneven. Selenium deficiency in soil and water can pose as much of a problem as excessive concentrations.

Selenium can be present in one of four oxidation states (-2, 0, +4, and +6). It commonly substitutes for sulfur in sulfide minerals (ATSDR 2003). In rocks, selenium tends to accumulate

in the carbonate fragments of sandstone, shale and slate, and phosphate rocks. Some of the highest selenium concentrations are found in carbonaceous shale and coal deposits (Wang and Gao 2001) and in sandstone-type uranium deposits (ATSDR 2003).

Chemical Forms of Selenium in the Environment

Weathering of parent rock releases selenium to soil and surface waters. In aquatic systems, inorganic selenium occurs primarily in one of two oxidation states, generally referred to as selenate, Se(VI), and selenite, Se(IV). Both valence states coexist in most surface waters, although Se(VI) is favored thermodynamically. Between pH 3.5 and 9, both selenate (SeO_4^{2-}) and selenite ($Se_2O_5^{2-}$) are present, with sodium as the predominant counter ion (Robberecht and Van Grieken 1982).

The average concentration of selenium in fresh water is reported to be 0.2 μg/L (Wang and Gao 2001), but naturally occurring concentrations can cover a wide range. In Finland, a selenium deficient area, a groundwater survey by Alfthan et al. (1995) showed that selenium in groundwater ranged from 0.0041 to 2.720 μg/L. The median selenium concentration of uncontaminated groundwater samples was 0.051 μg/L. In selenium deficient areas of China, concentrations in well water are as low as <0.2 μg/L. In contrast, the highest concentration in groundwater in a seleniferous area was 72,000 μg/L (Wang and Gao 2001).

Sodium selenate and selenite are soluble and mobile in water and soil solution, although their mobility is controlled by iron, manganese, and aluminum oxides in moist and semi-moist acidic soils, and by calcium, magnesium, and potassium oxides in semi-dry alkaline soils. Selenium in high organic content soils is effectively immobilized through adsorption to organic matter.

Selenite can be rapidly reduced to elemental selenium under mild reducing and low pH conditions. Reduction of selenate to selenite or elemental selenium is a slower reaction. Once formed, elemental selenium is stable and insoluble over a wide range of pH values and a range of mildly oxidizing to reducing conditions in soils and sediments.

Organic selenides are also present in natural surface waters. Organic selenides are typically assumed to be Se(-II) species in the form of free and combined selenomethionine and selenocysteine (Fisher and Reinfelder 1991). Dissolved organic selenides account for much of the dissolved selenium in fresh and marine waters and may be important to uptake by aquatic organisms (U.S. EPA 2004).

Selenium can be methylated by microorganisms in soil and sediment. Organic selenides are most readily methylated, followed by inorganic selenium and elemental selenium. Methylated selenium forms include dimethyl selenide and dimethyl diselenide, which are volatile. Microorganisms also mediate demethylation of selenium compounds in soils (U.S. EPA 2004).

Selenium concentrations in soils depend on the concentrations in the parent rock. The northwest, southeast, and Great Lakes states have low (<0.05 ppm) soil selenium concentrations because the soils in those areas were derived from volcanic deposits, glacial till, or well-washed coastal deposits. Soils originating from cretaceous shale, such as those found in South Dakota, Montana, Wyoming, Nebraska, Kansas, Utah, Colorado, and New Mexico, tend to have high (2 to 10 ppm) soil selenium concentrations. In particular, high selenium exposures that have been reported in people living on ranches in parts of Wyoming and South Dakota are most likely related to the elevated concentrations of selenium in native rock in these areas (e.g., >100 ppm in sandstone in Wyoming; >40 ppm in specific shales in South Dakota) (ATSDR 2003). Shacklette and Boerngen (1984) report the average selenium concentration in U.S. soil to be 0.39 mg/kg. Average county-by-county soil selenium concentrations for the conterminous United States are reported by the U.S. Geochemical Survey, and are shown in Figure 1.

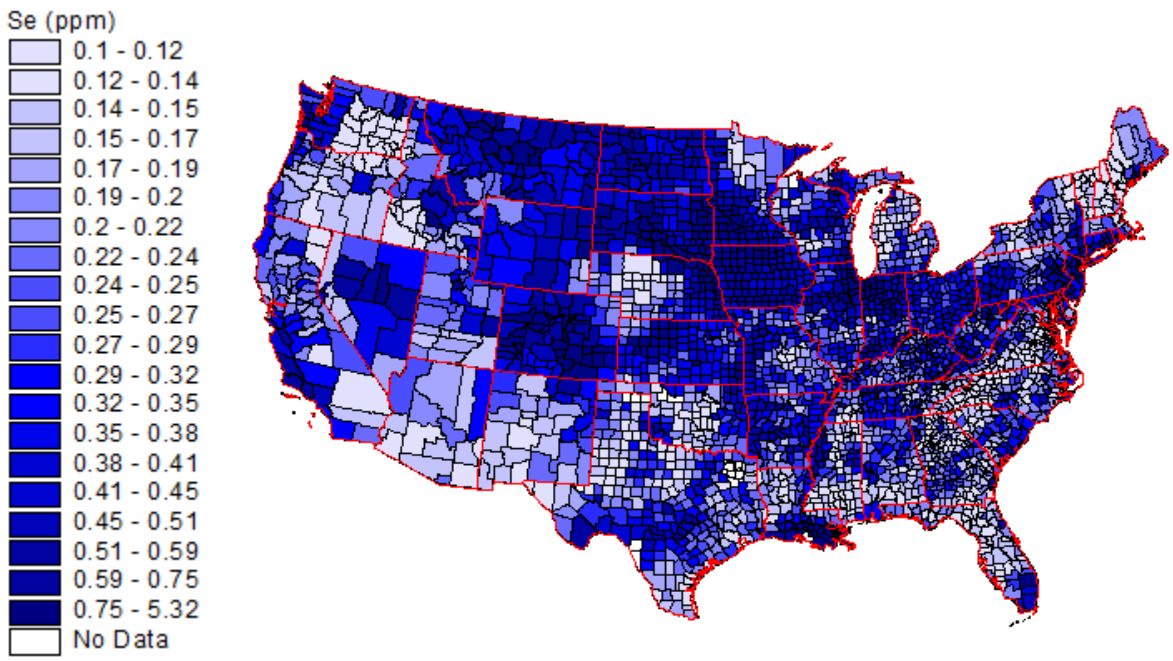

Figure 1. Selenium in counties of the conterminous United States from the National Geochemical Survey (USGS 2008)

General Characteristics of Coal Combustion Ash

Two types of ash are generated during coal combustion: bottom ash or slag, which falls into the bottom of the combustor, and fly ash, which is vented along with exhaust gases and collected with emission control devices such as electrostatic precipitators. Fly ash represents approximately 74 percent of the generated coal combustion products, bottom ash approximately 20 percent with the remainder being boiler slag (U.S. EPA 2009). Despite being of the same origin, bottom and fly ash differ in their physical and chemical characteristics. Fly ash consists of small glassy, spherical particles (Gierè et al. 2003; Tishmack and Burns 2004) that may be hollow and empty, or hollow and packed with smaller spherical particles (Tishmack and Burns 2004). Bottom ash, on the other hand, consists primarily of random conglomerations of fused subangular and angular particles (Tishmack and Burns 2004). Fly ash particles are several orders of magnitude smaller in diameter than bottom ash particles (Tishmack and Burns 2004).

Chemical compositions of fly and bottom ashes vary with coal origin and rank. However, both ash types are mainly composed of oxides of silica, aluminum, iron, calcium, magnesium, and

sulfur (Tishmack and Burns 2004). Ashes from sub-bituminous and lignite coals contain greater amounts of calcium and magnesium oxides, which are basic minerals that form basic solutions when mixed with water. Bituminous coal ashes, on the other hand, consist of higher amounts of pyritic iron than sub-bituminous or lignite coal ashes, which results in acidic solutions when mixed with water (Tishmack and Burns 2004). The "natural pH" of bituminous and sub-bituminous ashes ranges from 4.5 to 6.5 and from 10.6 to 12.3, respectively, when mixed with deionized water in a ratio of solid to liquid of 1:10 (Wang et al. 2007).

In addition to the major components, fly ash contains trace elements including arsenic and selenium whose concentrations in the ash are generally enriched relative to the original coal. Enrichment factors for trace elements in fly ash ranging between 2 and 100 times have been reported (Gierè et al. 2003).

Selenium in Coal Ash

Total selenium concentrations in different coal combustion products are summarized in a technical background document for report to Congress (Anonymous 1999). Total selenium concentrations in fly ash were reported to range from 0.13 to 49.5 mg/kg from different data sources (Anonymous 1999), although concentrations up to 200 mg/kg have also been reported (Wang et al. 2007 and references therein). Selenium concentrations in bottom ash, on the other hand, were reported to range from 0.007 to 14 mg/kg (Anonymous 1999). The median concentrations from different studies also suggest that selenium concentration in fly ash is higher than in bottom ash, which is a similar observation to that for arsenic. A 1999 report to Congress (Anonymous 1999) noted median selenium concentrations in fly ash between 5.5 and 10 mg/kg. In separate reports cited by Anonymous (1999), median concentrations in the bottom ash were 0.6 and 0.8 mg/kg.

Coal source does not appear to significantly affect selenium concentrations in coal ash. Selenium concentrations in fly ashes from bituminous, sub-bituminous and lignite coals appear to be within a similar range (Eary et al. 1990). On the contrary, other studies have measured higher selenium concentrations in bituminous than in sub-bituminous coal ash (Wang et al.

2007). In laboratory leaching studies, selenium has been found in an exchangeable fraction, bound to organic matter, or in residual fraction (Narukawa et al. 2005)

Leachability of Selenium from Coal Ash

Leaching of selenium from coal ash is dependent on many factors; however, the key factor appears to be the pH of the water that ash comes into contact with. Wang et al. (2007) investigated leaching of selenium from bituminous and sub-bituminous coals. For bituminous coals, selenium concentrations in leachate showed a parabolic relationship with pH. The smallest amount of selenium was released at a pH range from 3 to 4, while selenium leaching increased with increasing pH above 4 and decreasing pH below 3 (Wang et al. 2007). At pH 12, where the maximum amount of selenium leaching was observed, 55 to 69 percent of total selenium in coal ash was released. Compared to bituminous coal, leaching of selenium from sub-bituminous coal ash was low throughout the pH range from 2 to 12 (Wang et al. 2007). The high calcium content of sub-bituminous coal ash has been found to control leaching of arsenic and selenium by formation of calcium precipitates.

Selenium concentrations in laboratory leaching tests with water have ranged from 210 to 1,290 μg/L[1] (Narukawa et al. 2005). These amounts are similar to those measured in leachates from coal combustion product management sites. EPRI (2006) reported selenium concentrations ranging from 0.071 to 1,760 μg/L with a median of 19 μg/L for 67 ash leachate samples collected from landfills or impoundments that manage coal combustion products. The highest selenium concentrations were observed in leachates from landfills for sub-bituminous/lignite coal ashes, a result contrary to laboratory findings by Wang et al (2007).

In laboratory tests, the main selenium species in leachates is reported to be Se(IV) (Narukawa et al. 2005), a finding also reported by Wang et al. (2007). The main selenium species in leachates from ash ponds and bituminous coal ash was Se(IV). In contrast, Se(VI) dominated in sub-bituminous/lignite coal ash and in landfill settings (EPRI 2006).

[1] The aqueous concentration was reported as mg/kg.

Bioaccumulation and Trophic Transfer

Selenium is a food-chain contaminant and can biomagnify in the aquatic food chain up to 40,000 times higher than in water. Water concentrations remain in the parts per billion (ppb) range, even when severe effects are observed in aquatic biota. It is now understood that water concentrations are not predictive of whether selenium will bioaccumulate within a particular water body (Luoma et al. 1992). The initial movement of selenium from the water into sediment-associated particulate matter is dependent upon the species of selenium present and the turnover rate and redox potential of the sediment and lower portion of the water column. The type of benthic organisms present significantly affects the scale of biomagnification that will occur, as higher organisms (e.g., water column invertebrates and fish) bioaccumulate selenium only one- or two-fold above that of their prey. This section reviews what is known about the variables that drive selenium bioaccumulation.

Selenium Input and Loss

The rate at which selenium will accumulate in a system is partly a function of the relative loading and loss rates and partly a function of particulate sorption and uptake rates. Lentic systems, such as lakes or reservoirs or side channels and wetlands along rivers and streams, have long hydraulic residence times while lotic systems, such as fast-flowing streams or rivers, have much shorter hydraulic residence. Long hydraulic residence times combined with high selenium input over sustained periods contributes directly to a highly selenium enriched system as a result of increased loads. Belews Lake, North Carolina, a reservoir with long hydraulic residence time that was exacerbated by internal recirculation of selenium-contaminated water from coal ash disposal, is a well-known example of a lentic system highly enriched with selenium (Lemly 2002). At the other extreme, selenium loadings into high-gradient lotic ecosystems result in little accumulation in sediments and low rates of selenium bioaccumulation and biotransformation in main-channel areas (Adams et al. 2000).

Lentic systems also have higher biological and microbial activity than do lotic systems. Selenium from coal ash enters aquatic ecosystems primarily as selenate (Se VI) oxyanions on

particles (Jackson and Miller 1998), which may be dissolved into water, depending upon the pH of the system (solubility is maximized under alkaline conditions of high pH [Masscheleyn et al. 1991]). Conversion of selenate (Se VI) to selenite (Se IV) does occur through relatively slow thermodynamic processes, but in most aquatic systems, biological reactions are much more rapid and therefore are the primary drivers of conversion among selenium species and from dissolved to particulate forms (Oremland et al. 1989). Inorganic selenate and selenite are taken up by primary producers including aquatic macrophytes, algae, and phytoplankton, and converted to organic forms of selenium (Fan et al. 1997, 2002). Selenomethionine is the primary organic form of selenium formed at the base of aquatic food webs; it bioaccumulates up the food chain, with selenocysteine playing a minor role (Fan et al. 2002). Inorganic selenium species may also be taken up by reducing bacteria and converted from selenate to selenite or metabolized to form methylated selenium species that are highly volatile and may serve as a mechanism of loss of selenium from the system (de Souza et al. 2001; Fan et al. 1998; Peters et al. 1999; Turner et al. 1998).

Bioaccumulation

Bioaccumulation occurs when an organism takes up a chemical into its tissues, either from the food that it eats or directly from the environment (water, soil, sediment, or air). It is a necessary requirement for toxicity to occur. For some chemicals or some specific parts of the food chain, biomagnification occurs when the organism has higher concentration than its food, or in other instances biodilution may occur when higher trophic level organisms have lower concentrations. This section discusses how selenium is bioaccumulated by different groups of organisms in the aquatic food web.

Particulates

Because selenium enters the food chain through uptake by particulate matter, dissolved selenium must sorb to particulate matter to contribute to uptake and accumulation in the food web. The amount of partitioning that occurs is described by K_d, the partition coefficient (the ratio of selenium in particulate matter to selenium in the dissolved phase). Because the

composition of the particulate phase of an aquatic habitat is dependent upon the hydrology and microbial activity of the water body, the selenium K_d can vary widely and is highly dependent upon environmental conditions. Particulates consist of phytoplankton and attached algae, organic detritus, and microbial biofilms, as well as associated fine sediments. The speciation and rate of uptake of selenium in the water column will be maximized where there are higher amounts of living material (e.g., phytoplankton and microbes). Selenium is a required element for algae and phytoplankton (Price et al. 1987) and is taken up by active, energy-requiring processes; dead cells have little to no uptake (Fisher and Wente 1993). Different selenium species are taken up at different rates, with reduced forms (e.g., selenite) taken up more quickly than oxidized forms (e.g., selenate) (Riedel et al. 1991). Therefore, microbial processes that reduce selenium can significantly increase the K_d.

Because the uptake of selenium is an enzymatically controlled function and not a passive electrostatic binding, the degree of enrichment of algal cells is not directly related to water concentration and is, in fact, greater at lower concentrations. Increases in water concentrations result in nonlinear increases in phytoplankton concentrations (Baines and Fisher 2001). Additionally, rates of uptake vary greatly among algal taxa (over several orders of magnitude) even under similar environmental conditions (Baines and Fisher 2001), likely because of species differences in intracellular requirements. Species composition of algal communities varies with nutrient loadings, selective grazing pressure, and season. Thus, a body of water could have seasonally variable selenium concentrations that reflect changes in algal communities. Bacteria also bioconcentrate selenite out of water (Baines et al. 2004). Continual recycling and re-entrainment of particulates between the sediments and the water column enhances the uptake of selenium from the water column. Further, when cells lyse after the death of an organism (large or small), organoselenide is rapidly released back into the environment (Lee and Fisher 1992), and can be taken up by phytoplankton (Reidel et al. 1991). Degrees of enrichment can exceed 100-fold (Baines and Fisher 2001), indicating that this initial bioconcentration step (from the dissolved phase into living cells) is clearly a major determinant of selenium contamination in an aquatic food chain.

Invertebrates

Another critical driver for determining an overall site-specific K_d is the next step in the bioaccumulation process: uptake of selenium by sediment invertebrates that consume particulate matter. The uptake rates can be quantified through a biodynamic modeling approach using species-specific measures of intake rate, assimilation efficiency, growth rate, and elimination rate, as well as the concentration of selenium in the particulate matter (Luoma and Rainbow 2006); the amount of uptake of dissolved selenium is insignificant in comparison to the amount of selenium on particulate matter, and so is generally ignored (Luoma et al. 1992). The species-specific K_d (also known as the trophic transfer rate), varies several orders of magnitude among taxa (Stewart et al. 2004). Furthermore, assimilation efficiency by aquatic invertebrates varies from as low as 32.1% to as high as 92.3%, depending upon the invertebrate species and the type of phytoplankton on which they are grazing (Shlekat et al. 2002). Thus, the types of benthic invertebrates that are present in an ecosystem are critical determinants of the degree of biomagnification of selenium that will occur.

Vertebrates

Selenium bioaccumulation is much less in the higher trophic levels. Fish trophic transfer rates average approximately 1; that is, fish have nearly the same concentration of their prey items (Chapman et al. 2009), regardless of whether the fish are insectivorous or piscivorous. Clearly, the greatest amount of food chain enrichment with selenium occurs at the lowest trophic levels. This limits the amount of biomagnification that can occur, as compared to methyl mercury or persistent organic compounds that biomagnify along the entire length of the food chain, and accumulate the most in longer-lived species at the top of the food chain. Regardless, biomagnification between sediment and aquatic invertebrates can be sufficiently great in some ecosystems to reach concentrations that are toxic to fish and wildlife consumers. Because fish tissues will be equivalent in concentration to their invertebrate food, they too may become sufficiently contaminated to cause toxicity to their consumers. Whether or not this occurs is highly dependent upon the rate of input of selenium and the trophic structure (who is eating whom), which is the most important determinant of bioaccumulation within a food web (Stewart et al. 2004).

Selenium Toxicity

Cellular Mechanisms of Selenium Toxicity

Selenium had been thought to exert toxicity through substitution for sulfur. Reproductive toxicity and embryo malformations resulted when selenium displaced sulfur during embryonic development of structural proteins by disrupting the S-S linkages required for the normal protein tertiary structure (Diplock and Hoekstra 1976; Lemly 1997a). However, this theory has largely been discredited now that it is known that selenium is primarily incorporated into the amino acids selenomethionine and selenocysteine. The selenium moiety in selenomethionine is covered by the terminal methyl group of the amino acid structure and so does not alter either the structure or function of the proteins that substitute selenomethionine for methionine (Figure 2). The selenium moiety in selenocysteine is at the terminal end of the molecule (Figure 3). Moreover, it is now known that, unlike selenomethionine that is formed in a concentration-dependent manner, selenocysteine is highly regulated at the ribosomal level (Stadtman 1996). Proteins requiring selenium for their structure or function specify incorporation of selenocysteine, and there is no evidence that selenomethionine is randomly substituted instead (Allan et al. 1999).

Consequently, it now is generally accepted that oxidative stress is the primary cause of physical and biochemical effects of excess selenium (Spallhotz and Hoffman 2002; Hoffman 2002) despite its role as an antioxidant at lower doses. Selenium acts as an oxidant by reacting with cellular sulfhydryl groups. Excess selenium reduces heme biosynthesis by inhibiting blood aminolevulinic acid dehydratase and hepatic ferrochelatase enzymes, both of which require sulfhydryl groups (Padmaja and Prasad 1993). Interaction of selenium with glutathione also generates oxidative stress (Spallholz et al. 2004). Glutathione is a critical intracellular antioxidant, but can react with selenium to produce selenopersulfides (Spallhoz and Hoffman 2002) that spontaneously produce superoxide anions (Lin and Spallholz 1993) and thiyl radicals that react with glutathione to form glutathione disulfide radicals (Arteel and Sies 2001). Superoxide is highly toxic to cells, and increases mutagenesis and genetic instability, cell aging, and cell death.

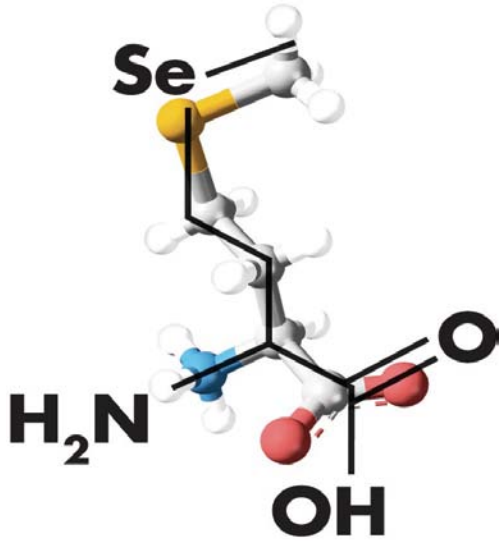

Figure 1. Selenomethionine

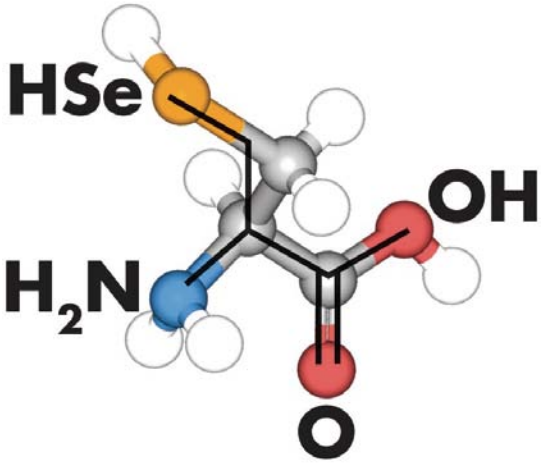

Figure 2. Selenocysteine

Hoffman (2002) reviewed the avian literature that documented that selenium exposure in birds changes the ratios of reduced to oxidized glutathione and increases lipid peroxidation and oxidative cell damage. Plasma and hepatic glutathione peroxidase activity also increases with increasing dietary selenomethionine (Hoffman et al. 1989, 1991, 1992a,b, 1996; Fairbrother and Fowles 1990). Glutathione peroxidase protects cellular membranes from cytotoxic effects of lipid peroxidation by using glutathione to detoxify lipid hydroperoxides to less toxic fatty acids (Berdanier 1998). Alterations in the ratio of reduced:oxidized glutathione restricts the ability of glutathione peroxidase to reduce lipid hyperperoxides. Similar results have been observed in rat (LeBoeuf et al. 1985) and fish models (Miller et al. 2007; Atencio et al. 2009).

Oxidative stress also may be involved in the pericardial and yolk sac edema observed in rainbow trout (*Oncorhynchus mykiss*) embryos exposed to elevated selenium (Palace et al. 2004). Selenium-exposed medaka (*Oryzias latipes*) have reduced hepatic activity of superoxide dismutase (SOD), an enzyme that neutralizes the effects of superoxide anions, converting them to hydrogen peroxide and molecular oxygen (Li et al. 2008). Teh et al. (2004) described histopathological lesions in the livers of splittail (*Pogonichthys macrolepidotus*) fed a selenized yeast diet, which the authors speculated could be caused by lipid peroxidation.

Toxicokinetics and Toxicodynamics

Maternal deposition of selenium into eggs and the subsequent assimilation of that selenium by the developing embryo are key to understanding how selenium causes deformities and reduces viability of embryos from egg-laying (oviparous) animals. This section briefly reviews what is known about deposition of selenium into eggs of fish, birds, reptiles, and amphibians.

Fish

Fish exhibit a range of reproductive strategies from species that spawn only once in their lifetime to species that spawn multiple times. Some species take many years to reach sexual maturity and spawn only every 2 to 3 years, while others spawn every year or even multiple

times each year (Rinchard and Kestemont 2005). It has been suggested that the timing and duration of oogenesis affects a species' susceptibility to selenium-induced reproductive toxicity.

In fish, the primary yolk precursor is vitellogenin, a phospholipoglycoprotein synthesized in the liver under the endocrine regulation of the hypothalamic-pituitary-gonadal-liver endocrine axis (Fairbrother 2000). Vitellogenin is exported from the liver, transported in the blood, and incorporated into the developing ovarian follicles where it is enzymatically cleaved into the primary yolk proteins (Fairbrother 2000). These sulfur-containing proteins can also contain selenium, and not surprisingly, selenium also has been demonstrated to bind to vitellogenin (Kroll and Doroshov 1991).

The amount and timing of vitellogenin deposition into developing oocytes depends upon the fish species and its reproductive strategy. Fish that spawn only once, such as salmonids, deposit vitellogenin over a period of several months prior to spawning (Estay et al. 2003). Fish that spawn multiple times within a season or year deposit vitellogenin close to the time the oocytes become mature, and in smaller amounts than salmonids (Rinchard and Kestemont 2005). Thus, repeat spawners will put the selenium that is in the diet at the time of spawning into their eggs, while salmonids are more likely to use stored selenium (e.g., from the liver). This makes it difficult to predict relative embryo toxicity from selenium across species with different reproductive strategies. It also becomes difficult to predict potential risks to salmonids from selenium exposures at the time of spawning, because of the delayed effect of accessing stored selenium. Life histories and prior selenium exposures need to be reconstructed (e.g., from laser ablation ICPMS of fish otoliths) to correctly evaluate risk at spawning sites.

Amphibians and Reptiles

Amphibians and squamate reptiles (lizards and snakes) incorporate vitellogenin proteins into oocytes in a manner similar to fish (Unrine et al. 2006). Oviparous amphibian eggs are very similar in structure to fish eggs, but vary tremendously in the number of eggs produced in each reproductive event. Some amphibian embryos develop in water where they can absorb additional selenium directly from water or contaminated sediments, while others develop in the

terrestrial environment (e.g., Plethodontid salamanders). Only one study has been published on maternal transfer of selenium (Hopkins et al. 2006), which showed that female narrow mouthed toads (*Gastrophryne carolinensis*) transferred selenium to their eggs in a nearly 1:1 correspondence of maternal:egg tissue concentrations.

In contrast, reptiles (turtles, crocodilians, lizards, and snakes) span an even broader range of reproductive strategies, from egg-laying to true viviparity (live births). Oviparous species produce an amniotic egg, more similar to that of birds rather than fish. Several studies have demonstrated maternal transfer of selenium in oviparous reptiles (alligator [Roe et al. 2004], lizard [Unrine et al. 2006]). Hopkins et al. (2005) and Unrine et al. (2007) showed transfer of selenomethionine from invertebrates to high trophic level lizards under controlled conditions, with partitioning of selenium to the developing follicles and to eggs. Selenium was transported to the egg by lipovitellin and two other egg proteins (Unrine et al. 2006) that contain selenomethionine and a compound that may have been selenocystine (Unrine et al. 2007).

Birds

In contrast to fish, amphibians, and reptiles, most of the selenium in an avian egg is found in the albumin and not in the yolk (Willhite 2006). This means that the egg selenium is metabolized before hatching and yolk sac resorption, so timing and development of terata (deformities) differ. This also means that reduced egg viability expressed as hatching failure among full-term fertile eggs is a highly sensitive endpoint for birds, although post-hatch toxic effects from yolk sac resorption can also adversely affect growth rates of newly hatched chicks and possibly cause direct chick mortality (Ohlendorf 2003).

In birds, most of the selenium in the eggs is deposited directly from the diet rather than from maternal tissues (Heinz 1996; DeVink et al. 2008). Consequently, under uniform dietary exposure, egg selenium is not related to laying order (Heinz 1996) and egg concentration reflects local and recent dietary exposure concentrations. Some clutches have highly variable selenium concentrations among the eggs as the hen forages for food from areas of differing

selenium concentrations. Hens also return to laying eggs with normal selenium levels within days to weeks of switching to a selenium-normal diet (Heinz 1996).

Physiological Requirements for Selenium

Selenium is a key component of a variety of functional proteins in all animals. There are three types of selenium-containing proteins: those that incorporate selenium nonspecifically, primarily as selenomethionine; proteins that specifically bind selenium; and enzymes that incorporate selenocysteine into their active site (Reilly 2006; Hesketh 2008). The selenium-containing enzymes glutathione peroxidase and thioredoxin reductase catalyze oxidation-reduction reactions; iodothyronine deiodinase activates or inactivates thyroid hormone; and selenophosphate synthetase mediates the synthesis of selenocysteine (Reilly 2006). In addition, there are more than 20 other selenoproteins identified in vertebrates whose functions are not yet known (Hesketh 2008).

Required Dietary Levels

Despite being an essential trace element, there is a very narrow margin between essentiality and toxicity in some species (U.S. EPA 2004) and, as a consequence, selenium uptake and excretion are tightly regulated. The recommended daily selenium intake in humans is 55-60 μg selenium per day (U.S. EPA 1991), a dose which is hypothesized to have primarily antioxidant and immune strengthening effects, and doses as much as 10-fold higher have been reported to have specific cancer preventive properties (Rayman 2005). By comparison, a lowest observable adverse effect level for toxicity has been reported at approximately 2-fold higher dose than this cancer preventive level (i.e., 1,260 μg/day; U.S. EPA 1991).

In comparison, channel catfish have been reported to require 0.1–0.5 mg Se/kg diet (wet wt) as inorganic selenium (assuming a feeding rate of 5 percent body weight per day, this is equivalent to 5–25 µg/kg-day) (Gatlin and Wilson 1984) and rainbow trout require a minimum of 0.7 µg/kg diet, equivalent to 3.5 µg/kg/day as sodium selenite (Hilton et al. 1980). More recent studies of juvenile grouper show a requirement of 0.7 mg Se/kg organic selenium (35 μg/kg/day

at 5% daily ration) for optimal growth (Lin and Shiau 2005). A selenium requirement in aquatic birds shows deficiencies observed below dietary concentrations of 0.3–1.1 mg Se/kg (Orhlendorf 2003), suggesting similar selenium demands in most vertebrates examined to date.

It appears that aquatic invertebrates and algae have much higher selenium requirements than vertebrates, although limited information is available. A single study with shrimp shows that 20 mg/kg selenium as inorganic selenium appears optimal (Yuchuan and Fayi 1993). Higher selenium requirements also have been documented for the unicellular freshwater green algae *Chlamydomonas reinhardtii* (3.9 µg/L) (Novoselov et al. 2002).

In birds, guidelines that have resulted from extensive testing with poultry are provided by Puls (1988). Dietary selenium concentrations of less than 0.3 mg/kg diet are considered to be below the range adequate for good adult health and reproduction and 3 to 5 mg/kg diet are high but tolerable. Concentrations lower than 1 mg/kg dw in eggs may indicate inadequate selenium in the diet (Puls 1988).

Health Effects of Selenium

People may be exposed to selenium in coal ash either through inhalation of coal ash dust, inadvertent ingestion of coal ash in soil, by eating grains or vegetables that have taken up selenium from coal ash in soil, or from fish that have accumulated selenium from coal ash in the river. Health effects of selenium has been reviewed extensively by the Agency for Toxic Substances and Disease Registry (ATSDR 2003) who reported that there is little information about inhalation effects of selenium and no reported non-occupational cases in the U.S. of poisoning from selenium dusts. Inhalation of selenium dusts in the form of selenium dioxides has been reported only in industrial workers in other countries, and causes respiratory tract irritation as evidenced by coughing, nasal discharge, nosebleeds, and loss of smell and headache (IPCS 1987). Symptoms are reversible following short term exposures, but chronic exposures (years) may result in continuing pulmonary dysfunction.

Only a few cases of selenium poisoning from oral exposure have been reported in the U.S., nearly all of which have been associated with industrial accidents; non-industrial cases include an error in manufacturing a selenium supplement that led to extremely high doses in consumers and a reported case of a family who drank well water contaminated with selenium (ATSDR 2003). Although selenium from coal ash released to the environment has in some cases been related to ecological effects, no cases of health effects in humans have been reported. This may be because, unlike mercury, selenium does not accumulate to very high levels in fish muscle tissue.

Selenium toxicity has been reported in people in China living in an area with unusually high naturally-occurring environmental levels of selenium in soil, water, crops, and coal used for heat and cooking. The lowest observable adverse effect level occurred at an intake rate of 1.26 mg/day (equivalent to 23 μg/kg-day for a 55 kg person in this population); the no observable adverse effect level was 0.85 mg/day (equivalent to 15 μg/kg-day for this population) (U.S. EPA 1991). This no-effect level is consistent with the maximum estimated dose of 0.724 mg/day and a lack of selenium toxicity reported for ranchers and their families in parts of Wyoming and South Dakota with elevated selenium in soils, forage, and livestock.

Signs of selenium poisoning include gastrointestinal upset, hair loss, thick and brittle fingernails and toenails, fatigue, irritability, and a characteristic garlic breath odor; extreme cases of selenosis can result in cirrhosis of the liver, pulmonary edema, neurological effects, and death (Yang et al. 1989; ATSDR 2003). Although excess selenium causes embryo malformations in birds and fish, there is no evidence of this occurring in people (Yang et al. 1989) or in studies with non-human primates (Tarantal et al. 1991), rats (Halverson et al. 1966; Rosenfeld and Beath 1954) or mice (Nobunaga et al. 1979; Schroeder and Mitchener 1971). Selenium is generally not considered a carcinogen and, due to its antioxidant (Shamberger 1985) and immunostimulatory (Koller et al. 1986) properties, is now thought to protect against prostate and certain other types of cancers (ATSDR 2003; Rayman 2005). High doses of selenium sulfide (the form used in anti-dandruff shampoos) have been found to increase tumor incidence in rodents when administered at high doses by oral gavage (bolus dosing) but not by dermal application (U.S. EPA 1991). Selenium deficiency is of more concern for human

populations than toxicity, as reduced selenium intake can negatively affect the thyroid, reproductive, and immune systems (ATSDR 2003).

How Health Risks of Selenium are Assessed

Human health risk assessments of environmental selenium exposure are conducted according to U.S. EPA risk assessment guidelines based on the non-cancer health effects of selenium. As noted above, the scientific evidence does not support an assessment of selenium from environmental exposures as potentially carcinogenic. To calculate a health-protective reference dose for oral exposure to selenium, U.S. EPA (1991) selected the no observed adverse effect level for the population in China of 0.015 mg/kg-day as a basis. This value was then reduced by a factor of three for protection of sensitive individuals, thereby resulting in an oral reference dose of 0.005 mg/kg-day (U.S. EPA 1991). This reference dose is considered to be protective of daily exposure for up to lifetime, including for sensitive individuals.

To assess the risks to people of environmental exposures to selenium, a total dose in mg/kg bodyweight per day for all sources of oral exposure would be compared to the oral reference dose. If the calculated dose exceeds the reference dose, then the potential exists for health effects to occur. The likelihood for effects would depend on whether long-term daily exposure actually occurs and the magnitude of the exceedance of this dose relative to the uncertainty factor of 3.

Ecological Effects of Selenium

The most prominent effects of selenium are deformities in embryos, larvae, and young birds. Failure to thrive (decreased weight gain) and increased embryo or larval mortality also may result from high selenium exposures. Adult mortality can occur, although it is rare. At low doses, selenium is an immunostimulant, but higher exposure levels may impair the immune system. This section briefly reviews effects associated with selenium toxicosis.

Deformities

The most prominent and well known toxic effects of selenium exposure of aquatic organisms are reproductive effects, especially deformities and decreased viability of embryos and early life stage organisms. Deformities include spinal curvatures, missing or deformed fins (fish) or limbs (birds), and craniofacial deformities, malformed bills (birds), and reduction or absence of eyes (birds), along with other toxicities such as edema (Hodson and Hilton 1983; Lemly 1993b; Maier and Knight 1994; Hamilton 2003; Hoffman and Heinz 1998; Ohlendorf et al. 1988). These terata are considered diagnostic for selenium toxicity (Lemly 1997a; Ohlendorf et al. 1989; Heinz 1996; Ohlendorf 2003; Seiler et al. 2003), with the exception of edema that can be caused by exposure to organic chemicals such as polycyclic and halogenated aromatic hydrocarbons (Grasman et al. 2004). Further, edema is not a true terata because it can be transient and reversible, and does not occur solely at the embryo-larval stage (Lemly 1993b). Nevertheless, there is a strong association between edema and elevated selenium concentrations in fish eggs (Holm et al. 2005; Muscatello et al. 2006; Gillespie and Baumann 1986; Pyron and Beitinger 1989) and it often is one of the most sensitive (Muscatello and Janz 2009) and prevalent endpoints (Woock et al. 1987; Holm et al. 2005; Gillespie and Baumann 1986).

Lemly (1997a) developed a scoring system and index of fish deformity rates, and stated that deformity rates of less than 5 percent are not ecologically relevant. This may be somewhat low as evidenced by field observations, and likely is species and location specific. Baseline deformity rates of 2–5 percent have been reported for hatchery-raised salmonids (Gill and Fisk 1966), with slightly higher rates for fish spawned in the wild (deRosemond et al. 2005; Kennedy et al. 2000; Holm et al. 2005). Villeneuve et al. (2005) reported deformity rates of several Cyprinids and a Catostomid species ranging from 6 percent in redside shiner (*Richardsonius balteatus)* to 17 percent in largescale sucker (*Catostomus macrocheilus)*. Deformities in northern pike (*Esox lucius*) were reported at less than 10 percent (Muscatello et al. 2006). It is estimated that up to 5 percent of fish produced in the aquaculture industry have some form of spinal deformity (Andrades et al. 1996).

Selenium does not affect fertilization success and hatching rates in fish (Gillespie and Baumann 1986; Coyle et al. 1993; Holm et al. 2005; Muscatello et al. 2006). However, in birds, impaired

egg hatchability occurs at lower egg selenium than embryo teratogenesis and is therefore a more sensitive effects endpoint (Skorupa 1999; Fairbrother et al. 1999; Ohlendorf 2003). Ohlendorf (2003) estimated the EC10 for egg hatchability from laboratory experimental data at 12 mg/kg in mallard eggs, while Seiler et al. (2003) reported an EC10 of 23 mg/kg for embryo deformities in field-collected duck eggs. However, compared to teratogenesis, impaired egg hatchability is a less selenium-specific and more easily induced response to many kinds of stressors; therefore, causation for impaired egg hatchability in the field can be more difficult to establish with high confidence.

Adult Mortality

Adult fish mortality can be caused by selenium at high and prolonged exposure levels. For example, Hermanutz et al. (1992) exposed adult bluegill sunfish (*Lepomis macrochirus*) to selenium in experimental streams dosed with selenite to achieve aqueous selenium concentrations of 10 and 30 μg/L. After 356 days, adult survival was reduced from 98.8 percent in the control fish to 83.8 percent in the 10 μg/L treatment; all the fish in the 30 μg/L selenium treated stream died. However, mortality of adult fish is rarely recorded as an effect endpoint because of the much higher sensitivities of embryos and early life stages. Similarly, much higher dietary exposure to selenium is required to induce adult mortality in birds than is required to cause embryo effects (Heinz 1996; Albers et al. 1996; O'Toole and Raisbeck 1997, 1998).

Immunotoxicity

As with other antioxidants, selenium has positive effects on the immune system at low dietary exposures (Koller 1986). Mammals have been shown to have a slightly reduced immune response at high dietary exposures of selenomethionine, sodium selenate, or sodium selenite (Raisbeck et al. 1998). Birds, however, are not as immunoresponsive to elevated selenium, although the organic form of selenium appears to be somewhat more immunoreactive than the inorganic form. Franson et al. (2007) showed increased immune responsiveness in eiders fed low dietary levels of selenium, and reduced activity of some parts of the immune system when dietary selenium was increased. Fairbrother and Fowles (1990) showed that selenomethionine

in drinking water decreased some aspects of the mallard immune response, while sodium selenite had no effect. Birds that hatch from eggs collected in selenium-contaminated areas show reduced responses in some parts of the immune system, but elevated activity in others (Fairbrother et al. 1994; Franson et al. 2007; Wayland et al. 2002) and an overall increase in mortality following a viral challenge (Fairbrother et al. 2004).

Factors that Modify Selenium Toxicity

Selenium toxicity is ameliorated or exacerbated by many environmental factors, including other trace elements, temperature, or nutritional factors.

Other Trace Elements

Interactions with other trace elements (e.g., arsenic, cadmium copper, lead, mercury, zinc) are generally antagonistic, although at extreme levels these antagonistic effects may be reduced or nonexistent (Stanley et al. 1994).

The most well-known antagonistic reactions are between organic mercury (methylmercury) and organoselenium. These two metalloids bind with each other in equimolar amounts to form metabolically inert mercury selenides (Ralston et al. 2006; Yang et al. 2008). The mutually protective effect of selenium and mercury on embryotoxicity that is observed when both metalloids are elevated simultaneously in diets has been recognized in poultry since the 1970s (El-Begearmi et al. 1977). The observed additive or synergistic effects of selenium and mercury in mallard embryos (Heinz and Hoffman 1998) despite antagonistic effects in adult mallards (Hoffman and Heinz 1998), remains unexplained. Antagonistic reactions between arsenic and selenium are also well documented (Levander 1977). Selenium and arsenic both increase the excretion of the other through the bile, possibly by forming a conjugate that is rapidly excreted by the liver (Levander 1977). Two studies examined the interactive effects of selenium and boron on mallards with opposite results; Hoffman et al. (1991) reported synergistic toxicity, while Stanley et al. (1996) did not. Fairbrother et al. (1994) showed no interaction of boron and selenium in avocet chicks.

Nutritional Factors

Diets with reduced protein levels increase selenium toxicity (Hoffman et al. 1991, 1992a,b) and high protein diets reduce selenium toxicity (Gortner 1940; El-Begearmi and Combs 1982) or have no effect (Hill 1979), possibly because different types of protein offer different levels of protection similar to what is seen with cationic metals, such as lead (Pattee and Pain 2003).

In contrast to the beneficial effects of increased dietary protein, excess dietary carbohydrate has been shown to enhance dietary selenium toxicity in rainbow trout (Hilton and Hodson 1983). The mechanisms of how proteins or carbohydrates influence selenium toxicity are unknown.

Winter Stress Syndrome

In aquatic ecotoxicology, the term “winter stress syndrome” has been proposed to describe the potential for pollutants to increase overwinter mortality (Lemly 1996). Fish are naturally at risk of overwinter mortality as a result of low temperature, low oxygen, reduced food availability, increased predation, disease, and parasitism (Hurst 2007). Winter stress is more severe in smaller fish because of size-dependent changes in surface:volume relationships (Post and Parkinson 2001). Survival beyond the first year of life is a critical factor in maintenance of a stock or population, and significant overwinter mortality of young-of-the-year can cause a recruitment bottleneck and negatively impact the size of the reproductive cohort (Post and Parkinson 2001; Hurst 2007).

Lemly (1996) noted three conditions for winter stress syndrome to occur: 1) the presence of a metabolic stressor (natural or anthropogenic), 2) cold water temperatures, and 3) reduced activity and foraging by the fish. Most importantly, Lemly (1996) noted that the potential for a stressor to lead to winter stress syndrome depends on its ability to increase metabolic rate and, therefore, energy demands.

The hypothesis that selenium can induce winter stress syndrome is supported by two laboratory studies that exposed juvenile bluegill sunfish for 180 days to dietary and waterborne selenium under either summer (20°C) or winter (4°C) temperature conditions (Lemly 1993a; McIntyre et

al. 2008). Winter conditions of low water temperature increased mortality, decreased condition factor (weight-to-length), and decreased lipid (energy stores). Lemly (1993a) suggested that selenium causes increased metabolism at least in part through oxidative stress.

However, two field studies in several native fish species inhabiting areas receiving complex metal mine effluents containing elevated selenium and other metals failed to support the winter stress hypothesis (Bennett and Janz 2007a,b; Kelly and Janz 2009; Driedger et al. 2009). Measures of growth (length, weight, condition factor, muscle RNA : DNA ratio, muscle proteins) and energy storage (whole body lipids, whole body triglycerides, liver triglycerides, liver glycogen) were determined in fish collected along gradients of exposure and from reference sites just prior to ice-on and immediately after ice-off. It was hypothesized that fish collected in spring from all sites (exposure and reference) would exhibit decreases in growth and energy storage measures compared to the previous autumn, and that these measures would be decreased to a greater extent in juvenile fish collected from exposure sites. However, fish collected from exposure sites exhibited similar or greater growth and energy stores in spring when compared to the previous autumn, and when compared to reference sites (Bennett and Janz 2007a,b; Driedger et al. 2009). Only slimy sculpin (*Cottus cognatus*) exhibited changes in energy stores (whole body triglycerides) that were consistent with the winter stress syndrome hypothesis (Bennett and Janz 2007b).

Evidence exists for winter stress in avian species as well. Heinz and Fitzgerald (1993) exposed mallards to diets supplemented with selenium for 16 weeks overwinter (November 16–March 7) in outdoor pens. Mortality occurred in a dose-dependent manner and no mortality occurred in the control or lowest dose levels. The authors noted that the majority of mortalities occurred during the period of December 26– January 11, when temperatures were consistently below freezing and all dead birds were extremely emaciated with no body fat. Other studies conducted during spring and summer months reported little to no mortality in selenium-treated mallards (Heinz et al. 1987, 1989) The authors argue that the increased energy demands of cold weather probably force ducks to eat more selenium-treated food, thus leading to effects faster and at lower concentrations than would occur in warm weather.

Selenium Toxicity Threshold Values

Lemly (2002) states that food chain organisms remain unaffected even when they accumulate relatively high selenium body burdens, so only fish and higher order vertebrates are at risk. However, DeBruyn and Chapman (2007) concluded that selenium may cause toxic effects in some freshwater invertebrate species at concentrations considered safe for their predators, and recent studies have shown that all species are subject to adverse effects from elevated selenium exposures. Response thresholds differ significantly among taxa. This section reviews the literature on comparative sensitivity of aquatic organisms at all trophic levels and discusses the U.S. Environmental Protection Agency's (EPA's) proposed revision to the selenium water quality criteria.

Bacteria

Bacteria are tolerant of metals and metalloids in general. This may stem from the ability of some bacteria to sequester selenite in insoluble nodules (Sarret et al. 2005). In aquatic systems, bacteria can accumulate twice as much selenium without apparent impairment as do phytoplankton (Baines et al. 2004).

Algae

Algae also can be tolerant of selenium, and can acquire relatively high tissue burdens without any measurable adverse effects (Kiffney and Knight 1990). Accumulation can range by four to five orders of magnitude, depending upon the algal species, when exposed to 0.5 to 4.5 nM selenite (Baines and Fisher 2001). Chlorophytes have the lowest selenium enrichments, while prymnesiophytes, prasinophytes and dinoflagellates have the greatest enrichments. Only one species of diatom has concentrations that vary in proportion to environmental concentrations (Baines and Fisher 2001). This suggests that algae actively regulate internal selenium loads, as would be expected for an essential nutrient. They can efficiently volatize selenium as dimethylselenide particularly when sulphates are limiting (Neumann et al. 2003), sequester it as non-protein selenoaminoacids such as methylselenocysteine or selenocystathionine (Brown and Shrift 1982), or accumulate it in an insoluble form as elemental selenium (Se^0), which has a

relatively low toxicity (Lortie et al. 1992). The high tolerance and biomagnification of algal species for selenium is of concern because they can reach concentrations that are toxic to consumers in higher trophic levels (e.g., fish) (Baines et al. 2001). However, some algae such as the green alga *Scenedesmus quadricauda* can be relatively sensitive to dissolved selenium. *S. quadricauda* died at selenium concentrations of > 50 µg/L selenate or selenite, and had significantly slower growth at selenite concentrations as low as 10 µg/L (Umysová et al. 2009).

Protozoans

There is a high degree of uncertainty about toxicity of selenium to protozoans, as the database is very small. Bovee (1978) reported effects to the ciliate, *Tetrahymena pyriformis,* at selenium water concentrations as low as 5 µg/L (reduced swimming speed), but suggested that the threshold for ecologically-relevant growth and survival effects would be 20 µg/L selenium. Conversely, Sanders and Gilmour (1994) reported that population growth rates of the ciliate *Paramecium putrinum* were not inhibited until selenium concentrations (as dissolved selenite or selenate) exceeded 1,000 µg/L. Pratt and Bowers (1990) reported an intermediate threshold of 80 µg/L for protozoan species richness in laboratory microcosms.

Macroinvertebrates

U.S. EPA (2004) ranked aquatic invertebrates as the most sensitive species compared to fish for acute exposure to aqueous selenite and selenate. For selenite (Se IV), *Hyalella* spp. are the most sensitive, with a Genus Mean Acute Value (GMAV) LC50 of 461.4 µg/L. *Ceriodaphnia* spp. are the second most sensitive, with a GMAV of 515.3 µg/L. *Daphnia magna* Species Mean Acute Value (SMAV) is 905.3 µg/L; when the single *Daphnia pulex* study is included, the GMAV increases to 1,341 µg/L. There is one study of *Hydra* spp. with an LC50 of 1,700 µg/L. *Gammarus* spp. are among the least sensitive (and is less sensitive than two species of fish), with a GMAV of 3,489 µg/L. The leech, *Nephelopsis obscura,* has the highest SMAV of

203,000 μg/L. For selenate (Se VI) (adjusted for sulfate concentration[2]), invertebrates are both the most sensitive and the most tolerant freshwater species to selenate, with sulfate adjusted SMAVs ranging from 593 μg/L for *Daphnia pulicaria*, to 1,515,616 μg/L for the leech (U.S. EPA 2004).

U.S. EPA (2004) also summarized toxicity thresholds from chronic exposures. Acceptable freshwater chronic toxicity data are available for only a single aquatic invertebrate, *Brachionus calyciflorous*, a freshwater rotifer. The study by Dobbs et al. (1996) exposed rotifers to sodium selenate in water and algae for 25 days. Rotifer growth was reduced at >108.1 μg/L in the water and the population died after 6 days in >202.4 μg/L (40 μg/g dry weight in the algae). This was equivalent to an EC20 of 42.4 μg/g dry weight tissue.

The summary findings of EPA for acute and chronic toxicity for aquatic invertebrates are in agreement of those of DeBruyn and Chapman (2007), who reviewed 156 studies of aqueous, dietary, or internal selenium concentrations associated with toxic effects in 29 macroinvertebrate species and found highly variable responses. They reported that water concentrations associated with acute lethality varied more than 1,000-fold among taxa, whereas toxic dietary concentrations varied about 100-fold and toxic internal concentrations varied about 30-fold. Sublethal effects occurred at 1−30 μg/g dry weight invertebrate tissue. The authors concluded that aquatic invertebrates are affected by internal selenium concentrations that are at the same levels that cause toxic effects in some fish species that consume them.

Fish

U.S. EPA (2004) summarized the acute toxicity to fish exposed to selenite (Se IV) in the water. Fish were less sensitive than some invertebrate taxa, with striped bass (*Morone saxatilis*) being the most sensitive fish species (GMAV of 1,783 μg/L) and the fourth most sensitive species overall. Fathead minnow (*Pimephales promelas*) had a GMAV of 2,209 μg/L. All other fish were less sensitive, with GMAVs ranging from 6,500 μg/L (*Jordanella* spp.) to 28,500 μg/L

[2] Selenate concentrations were regressed on sulfate concentrations for a tested species for which the data were reported. The slope of 0.5812 was used to adjust the freshwater selenate acute values to a sulfate level of 100 mg/L to standardize responses.

(bluegill). Fish also are less sensitive than invertebrates to acute exposures of selenate (Se VI) in water. The lowest GMAV reported by U.S. EPA (2004) is 10, 309 μg/L for the razorback sucker (*Xyrauchen texanus*). Channel catfish (*Ictalurus punctatus*) are among the least sensitive fish, with a sulfate adjusted GMAV of 226,320 μg/L. These concentrations of selenium, either as Se IV or Se VI, are rarely observed in natural systems, suggesting that acute toxicity of selenium in water to fish rarely (if ever) occurs.

However, chronic exposure through the dietary route can result in significant toxic effects at environmentally realistic concentrations. Because the primary route of chronic exposure to selenium is in the diet, U.S. EPA (2004) reviewed studies where exposure was through the diet alone, or diet plus water. Additionally, EPA chose to use whole-body tissue concentration of selenium on a dry weight basis as the medium upon which to base a chronic criterion. Using a dietary threshold concentration was considered inappropriate because of the variability of fish feeding habits and the difficulty in selecting an appropriate species upon which to base the criterion. A sediment-based criterion (as proposed by Canton and Van Derveer 1997; Van Derveer and Canton 1997) also was rejected by EPA because of the patchiness of selenium sediment concentrations and the site-specificity (as a result of species differences) of bioaccumulation of selenium from sediments into the food web.

The difficulty in setting a diet-based criterion is exemplified by the range of suggested values presented by several authors. Lemly (2002) summarized dietary toxicity thresholds for fish. Mortality thresholds were reported at concentrations as low as 3 μg/g dry weight in rainbow trout and as high as 50 μg/g dry weight in bluegill. Diet thresholds for reproductive failure were measured only in bluegill and ranged from 6.5 μg/g to 16 μg/g, depending upon the study. Lemly (2002) recommends 3 μg/g dry weight as a dietary toxicity threshold for fish. DeForest et al. (1999) concluded that a dietary EC10 of 10 μg/g dry weight was the most appropriate threshold value for selenium management purposes, based on the amount, range, and reliability of the data. Skorupa et al. (1998) recommends 3–8 μg/g dry weight.

Tissue concentration thresholds associated with mortality in four species of adult fish, as summarized by Lemly (2002), range from 1 μg/g dry weight (whole body) to 34 μg/g dry

weight (whole body), depending upon species and test conditions. Reproductive thresholds based on three fish species range from 18 to 24 μg/g dry weight (whole body). Growth of juveniles is slowed at 2 μg/g dry weight and reproductive failure occurs beginning at 19 to 23 μg/g whole body dry weight. Lemly (2002) recommends a tissue threshold concentration of 4 μg/g dry weight.

U.S. EPA (2004) calculated a final chronic value (FCV) for selenium of 7.91 μg/g dry weight whole body tissue concentration. In their review, EPA evaluated data from eight different fish species, and a mix of fish species from the family Centrarchidae, based on 21 distinct studies. For all endpoints taken together, bluegill are the most sensitive, with a GMCV of 9.5 μg/g dry weight (whole body) and the razorback sucker the least sensitive (GMCV = 23.28 μg/g dry weight, whole body). None of the studies reviewed by EPA reported adult mortality. The lowest effect level for juvenile mortality was 7.91 μg/g dry weight (whole body of juveniles) in bluegill subjected to winter stress, for juvenile growth was 10.47 μg/g dry weight (whole body of juveniles) in Chinook salmon (*O. tshawytscha*), and for larval deformities was 5.79 μg/g dry weight (whole body of parents) in rainbow trout.

The difference between Lemly's suggested tissue criterion of 4 μg/g dry weight (whole body) and EPA's proposed value of 7.91 μg/g dry weight (whole body) stems from interpretation of the Lemly (1993) winter stress study and EPA's repetition of that work (McIntyre et al. 2008). Although the accumulation of selenium in the fish was similar between the two studies, Lemly (1993) observed 40 percent mortality at a concentration of 5.85 μg/g dry weight (whole body) at the end of his study, while McIntyre et al. (2008) saw no mortality up to 10 μg/g dry weight (whole body). Furthermore, Lemly (1993) documented changes in a body condition factor based on amount of lipids and phosphorus present, whereas McIntyre et al. (2008) did not. Although the study designs were similar, there were two critical differences that confound a direct comparison: Lemly (1993) removed fish periodically for weighing, which McIntyre et al. (2008) did not, and Lemly (1993) had a shorter photoperiod than McIntyre et al. (2008). Repeated weighing may have caused additional stress to the animals, decreasing their body condition index and increasing mortality. Conversely, a longer photoperiod may have provided

more time for feeding, thus allowing the fish to maintain body condition that otherwise would have been depleted.

Because EPA has concerns about some aspects of Lemly (1993), and because McIntyre et al. (2008) did not completely corroborate Lemly's findings, EPA has not decreased their proposed criterion to the 4 μg/g dry weight (whole body) proposed by Lemly (2002). EPA recognized that the 7.91 μg/g value is lower than the lowest GMCV, so it already includes a degree of conservatism greater than what usually is included in an FCV calculation. However, EPA has added a caveat that if fish tissue samples exceed 5.85 μg/g during summer or fall, fish should be monitored during the winter to determine if selenium exceeds 7.91 μg/g. U.S. EPA (2004) verified that the proposed criterion was not below the natural background levels in fish by analyzing data from the National Contaminant Biomonitoring Program (NCBP) and the National Water Quality Assessment (NAWQA) dataset. The NCBP measured selenium in 591 fish samples representing 60 different species, and had a mean ($\pm$ standard deviation) of 1.9 ± 1.4 μg/g dry weight. NAWQA data included 90 fish samples with a mean ($\pm$ standard deviation) of 3.22 ± 2.29 μg/g dry weight. Thus, the FCV would not be exceeded by more than 97 percent of the fish samples collected. A criterion of 4 μg/g dry weight would be at about the 75th percentile of the background tissue concentrations, which would make application of a criterion difficult as it would be exceeded by 25 percent of fish in areas with background concentrations of selenium.

Amphibians

There are few studies on effects of toxics to amphibians, and nearly all the studies of selenium effects included simultaneous exposures to other metals in field studies. Therefore, causal relationships between selenium exposure and effects have been difficult to establish. However, Unrine et al. (2007) showed that tadpoles are extremely efficient at accumulating selenium in their tissues and Hopkins et al. (2006) demonstrated that narrow mouthed toads (*Gastrophryne carolinensis*) can transfer high amounts of selenium into their eggs (up to 100 μg/g in the egg). Amphibian larvae with high selenium concentrations in their tissues (20–28 μg/g dry weight) can show the same type of axial or craniofacial malformations seen in fish (Hopkins et al.

2000), experience reduction in hatching, and exhibit abnormal swimming behavior (Hopkins et al. 2006). Unlike other trace elements, selenium is retained (but not increased) as the amphibian undergoes metamorphosis (Snodgrass et al. 2003).

Birds

Reproductive impairment is considered to be the most sensitive indicator of selenium toxicity to birds (Ohlendorf 2003; Seiler et al. 2003). As in other oviparous species, birds transfer selenium to the egg with resulting embryo deformities and reduced viability. Egg selenium concentrations have been related to both hatchability and teratogenesis (Fairbrother et al. 1999; Ohlendorf 2003; Seiler et al. 2003).

All duck species appear to be equally sensitive to egg selenium (Seiler et al. 2003), with effect thresholds of >12 μg/g dry weight egg selenium. Black-necked stilts (*Himantopus mexicanus*) are less sensitive than duck, and American avocets (*Recurvirostra Americana*) are less sensitive still (Seiler et al. 2003). The EC50s for teratogenic effects are 30, 58, and 105 μg/g selenium in the egg for ducks, stilts, and avocets, respectively (Seiler et al. 2003). These data show that black-necked stilts are about twice as sensitive as avocets, and that ducks are about 3.5 times as sensitive as avocets to the teratogenic effects of selenium.

However, egg hatchability is a more sensitive endpoint in birds than teratogenesis. Multiple studies have been conducted with captive mallards fed diets containing various levels of selenium (Heinz et al. 1987, 1989; Heinz and Hoffman 1998; Stanley et al. 1994, 1996). Data from these studies show that a 10 percent hatch failure rate corresponds with egg selenium concentrations in the range of 12–15 μg/g (Adams et al. 2003; Ohlendorf 2003). Field studies suggest that the egg-based threshold for reproductive impairment in stilts may be either slightly lower (6–7 μg/g; U.S. DOI 1998) or slightly higher (21–31 μg/g; Adams et al. 2003) than the threshold in mallards. However, the American avocet is clearly less sensitive, as field-measured hatchability does not begin to decline until selenium concentrations in eggs exceed 60 μg/g (U.S. DOI 1998). Black-crowned night herons (*Nycticorax nycticorax*) also are less sensitive than mallards as there were no embryo effects at mean egg selenium concentrations of 16.5 μg/g

(Smith et al. 1988). Red-winged blackbirds (*Agelaius phoeniceus*), a common songbird in the western U.S., has a threshold for reproductive impairment of approximately 22 μg/g (Harding 2008). Conversely, the spotted sandpiper (*Actitis macularia*) may be one of the most sensitive species studied to date, as hatchability of eggs was 15 percent lower when selenium concentrations in their eggs averaged 7.3 μg/g in a selenium-contaminated area, than in a reference area where egg selenium concentration averaged 3.8 μg/g (Harding et al. 2005).

Mammals

Aquatic-dependent mammals rely on the aquatic food chain and include species such as mink (*Mustela vison*), otter (*Lutra canadensis*), muskrat (*Ondatra zibethicus*), raccoon (*Procyon lotor*) and beaver (*Castor canadensis*). Clark (1987) measured selenium in 10 species of mammals from the selenium-contaminated Kesterson National Wildlife Refuge and a nearby reference site. All species concentrated selenium in their livers. Average liver selenium concentrations for Kesterson organisms were as high as 32.1 mg/kg (dry weight) while animals from the reference area had liver concentrations <1.5 mg/kg. In 2005, Gamberg et al. (2005) measured selenium concentrations in mink livers from non-contaminated areas of the Canadian Yukon, with an average of 4.5 mg/kg (dry weight). However, no evidence of negative impacts on the organisms was found, even at the high concentrations found in animals from the Kesterson National Wildlife Refuge. Therefore, aquatic-dependent mammals appear to be considerably less sensitive than birds or fish to the effects of selenium. It should be noted, however, that the dataset for mammals is not as robust as for the other classes of animals, particularly in regard to potential effects on growth or reproduction.

System Recovery

Fish populations and communities can recover from selenium stress once the input to the system is stopped or significantly reduced. The best example of this is Belews Lake, North Carolina, where the fish community was severely impacted by selenium contamination from a coal ash discharge that began in 1975, with the loss of 16 species out of 20 originally present within one year (Lemly 1985). Selenium input continued until the early 1980s, during which time diversity

ranged from 7 to 13 taxa and biomass was estimated to be from 5.67 to 15.02 kg/ha (Barwick and Harrell 1997). Inputs ceased in 1985, and natural sedimentation covered the ash that had previously been discharged. Both diversity and biomass significantly increased following cessation of ash discharge (from 14 to 22 taxa and 36.39 to 79.66 kg/ha) with selenium-sensitive species such as bluegill and largemouth bass returning to the system by 1994 (Barwick and Harrell 1997). Fish populations in Belews Lake had generally recovered by the mid-1990s (Finley and Garrett 2007). Interestingly, whole body concentrations of selenium did not recover as quickly. Finley and Garret (2007) reported that median whole body selenium concentrations in carp (Cyprinidae), redear sunfish (*Lepomis microlophus*), and crappie (*Pomoxis* sp.) were approximately 22, 17, and 18 μg/g dry weight, respectively, in 1994–1996 and decreased to 9, 10, and 9 μg/g dry weight in 2004–2006, still above the average background concentrations of selenium. Additionally, the species composition of the fish assemblages is markedly different from that which was present during pre-exposure conditions (Lemly 1997b), although some argue that this is a consequence of the natural progression of species in reservoirs as they age over time.

Kesterson National Wildlife Refuge in California, which was severely impacted by selenium-contaminated agricultural drainwater in the early 1980s, was remediated in the 1990s. Agricultural drainwater input was terminated and the ponds were filled to turn the wetland into an upland ecosystem. Post-remediation monitoring indicated that continued exposures from ephemeral pools and terrestrial habitats continue to present a low-level of risk to wildlife (Ohlendorf 2002).

In another example, selenium was added to a series of 11 Swedish lakes in 1987 to ameliorate the effects of elevated mercury. Selenium concentrations in perch (*Perca fluviatilis*) increased from 0.8–2 μg/g whole body prior to selenium addition, to 6–36 μg/g in one year. By the second year, stocks from four of the lakes had disappeared (Skorupa 1998). However, the perch populations rapidly recovered once the selenium water concentrations were reduced to <2 μg/L. This example illustrates the rapidity with which selenium can affect a system when it is introduced, and the equally rapid recovery rates once inputs cease.

Based on demonstrated cases, aquatic populations have the capacity to recover in a relatively short time-frame (years, but not decades) following the cessation of aqueous selenium inputs. However, because selenium is accumulated in aquatic food webs as a result of sediment-based processes, full recovery will be dependent upon the rate at which full removal (or burial) of selenium in sediments occurs.

Summary

Selenium is a naturally occurring element that can bioconcentrate in organisms at the base of the aquatic food web to levels that are toxic to their consumers. Selenium is present in coal ash, with higher amounts found in fly ash than in bottom ash. It is soluble, and leaches from ash into water at rates that are dependent upon the pH of the water. Selenium is found in the environment in several oxidized states, with the most reduced form (selenide) forming a stable constituent of sediments. Selenium in water may become methylated and volatilized, which can be a significant loss mechanism for some systems. Selenite is more readily taken up from the water column by algae, microbes, and other particulates than is selenate. The rate of uptake into the aquatic food web through particulate matter and invertebrates is much greater in lentic (slow moving) systems than in lotic (fast moving) waters, and is highly dependent upon the types of invertebrates present. Fish and higher organisms do not concentrate selenium, so their body burden remains approximately the same as that of their prey (invertebrates and/or algae).

Selenium is primarily a reproductive toxicant in all fish and avian species, causing embryo death and deformities, and increased mortality of young, although such effects have not been reported in mammals are not a concern for human exposures. Selenium also is an immunostimulant and an anti-oxidant at low levels and can protect against some forms of cancer. At high exposure levels, selenium poisoning causes hair loss, brittle nails, fatigue and more severe systemic effects. These symptoms have been reported for people living in an area of China with usually high exposure from selenium in soil, water, the food chain, and coal used for fuel, but have never been reported in the U.S. Human health effects, such as respiratory problems have only been reported for workers inhaling selenium dioxide in dusts and the only known oral

poisonings in humans in the U.S. are from accidental poisonings or overdoses with dietary supplements.

Because of the reproductive risks to fish and avian wildlife from selenium-contaminated water, the EPA has set a water quality criterion for selenium. However, the site-specific nature of uptake and accumulation of selenium in the food web and the resulting inability to predict risk based solely on selenium water concentrations, has led EPA to publish a draft updated criterion for selenium based on whole body tissue concentrations in fish. Cold weather and/or food stress may exacerbate the effects of selenium on young-of-the-year, so EPA included a caveat in the proposed criterion to account for winter stress syndrome. Due to its relatively high degree of mobility in both the environment and biota, systems can recover from selenium stress relatively quickly (years, not decades) once the input has been reduced.

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