# 3 What You Need to Know about Selenium

*Terry F. Young, Keith Finley, William J. Adams, John Besser, William D. Hopkins, Dianne Jolley, Eugenia McNaughton, Theresa S. Presser, D. Patrick Shaw, Jason Unrine*

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## 3.1 INTRODUCTION

In 1976, scientists monitoring North Carolina's Belews Lake were perplexed by the sudden disappearance of the young-of-the-year age class of popular game fish species. This man-made reservoir was fed in part by water from a coal ash settling basin. By 1977, only 3 of the lake's 29 resident species remained. The

culprit was determined to be elevated concentrations of selenium (Se) in the food web. Across the country in 1982, federal biologists observed the local extinction of most fish populations in California's Kesterson Reservoir, a wetland area fed by agricultural drainage. They also discovered unnaturally high numbers of dead and deformed bird embryos and chicks. The multiple embryo deformities were sufficiently distinctive to be labeled the "Kesterson syndrome" (Skorupa 1998). Here too, Se was found to be the cause of the devastating impacts to the local ecosystem.

Selenium, however, is not a problem of the past. Se contamination of aquatic ecosystems remains a significant ecological issue of widespread concern, largely because Se is a common by-product of several core economic activities: coal-fired generation of electricity; refining of crude oil; mining of coal, phosphate, copper, and uranium; and irrigated agriculture. Because these industries are likely to continue and grow into the foreseeable future, the potential for large-scale, globally distributed Se contamination of ecological systems is likely to increase.

Since the discovery of the adverse environmental impacts of Se, our ability to identify, quantify, and limit the ecological risk of Se has grown and continues to expand. Starting with the work done at Belews Lake and Kesterson Reservoir, a significant body of research has grown regarding the transport, transformation, and effects of Se in the aquatic environment. We now know that

- Se is distributed globally in organic-rich marine sedimentary rocks,
- most forms of dissolved Se can be transformed and incorporated into food webs,
- organic forms of Se are the most bioavailable,
- the primary route of exposure to Se in consumer animals is via the food web rather than directly from water, and
- maternal transfer of Se to embryos causes reproductive impairment in egg-laying vertebrates.

Although many questions remain, the knowledge we have accumulated during the past 3 decades allows us to assess, predict, and potentially prevent the adverse ecological effects of Se with some confidence.

This chapter 1) provides an overview of the current understanding of Se interactions and impacts, with particular reference to the case studies that are summarized in Appendix A; 2) synthesizes these findings into a conceptual framework that incorporates Se sources, transport and transformation in nature, bioaccumulation and trophic transfer, and effects on ecological systems; 3) uses this conceptual framework to identify strategies for assessing potential Se problems in the field; and 4) recommends key areas for future research. These 4 organizing elements are drawn from the "Problem Formulation" step of the US Environmental Protection Agency (USEPA 1992) Ecological Risk Assessment Guidelines (Text Box 3.1). This chapter provides both an introduction to and a context for the more detailed discussions presented in later chapters.

**TEXT BOX 3.1 INITIATING AN ECOLOGICAL RISK ASSESSMENT FOR SELENIUM: PROBLEM FORMULATION**

In ecological risk assessments, the Problem Formulation step is designed to help define the nature and extent of the problem, the resources at risk, the ecosystem components to be protected, and the need for additional data to complete the assessment. The Problem Formulation step is often the most important step in the risk assessment process because it identifies the ecosystem attributes to be protected, identifies existing information and data gaps, and provides a means for consensus building between stakeholders for developing an analysis plan. The Problem Formulation step frequently contains 4 main elements, including 1) a synthesis of available information, 2) a conceptual model, 3) assessment endpoints that adequately reflect management goals and the ecosystem they represent, and 4) an analysis plan, which provides the details on data to be collected for risk management decisions (USEPA 1992; Reinert et al. 1998). The conceptual model is intended to identify key features of the ecosystem and resources to be protected and the stressors and the adverse effects that may result. The conceptual model helps identify the hypotheses to be tested during the analysis phase of the assessment.

## 3.2 WHAT IS SELENIUM (Se)?

The element Se is in the 4th period of group 16 (chalcogen group) of the periodic table. It has an atomic number of 34 and an atomic mass of 78.96 (Lide 1994). Se is chemically related to other members of the chalcogen group, which includes oxygen, sulfur, tellurium, and polonium. Selenium is classified as a non-metal, but elemental Se has several different allotropes that display either non-metal (red Se, black Se) or borderline metalloid or non-metal behavior (grey Se, a semiconductor) (McQuarrie and Rock 1991; Lide 1994). Unlike metals or transition-metals, which typically form cations in aqueous solution, Se is hydrolyzed in aqueous solution to form oxyanions, including selenite ($SeO_3^{-2}$) and selenate ($SeO_4^{-2}$). Oxyanions typically have increased solubility and mobility with increasing pH, in contrast to metals, which show the opposite behavior.

Recognizing the non-metallic behavior of Se is one of the keys to a better understanding of its geochemical behavior, but biologically mediated reactions dominate in ecosystems, where Se effects can be beneficial and detrimental (Text Box 3.2). Speciation and biotransformation are widely recognized as playing important roles in determining Se's fate and effects in the environment. Given the richness of biochemical pathways through which Se may be metabolized, it is important to understand the Se biotransformations that may occur in organisms and how they relate to bioavailability, nutrition, and toxicity.

### TEXT BOX 3.2 SELENIUM ESSENTIALITY AND TOXICITY

Swedish chemist Jöns Jacob Berzelius is credited with discovering Se in 1818 as a by-product of sulfuric acid production. Berzelius hypothesized that symptoms of toxicity presented by workers in his sulfuric acid factory were due to an impurity present in the pyrite ore used as a production feedstock. Ultimately Berzelius demonstrated that this impurity was an unknown chemical element and named it selenium, from "selene," the ancient Greek word meaning moon (Lide 1994; Wisniak 2000).

In the western United States during the 1930s, Se was identified as the toxic factor of alkali disease in cattle and livestock (Trelease and Beath 1949; Anderson et al. 1961). The US Department of Agriculture conducted both controlled experiments and broad geographic surveys of soil and plant Se to assess the toxic hazards and risks associated with environmental Se. Open-range forage plants included Se accumulator plants of the genus *Astragalus* growing on the Peirre Shale that contained Se concentrations of up to 10,000 mg/kg dw (Trelease and Beath 1949; Anderson et al. 1961). Yang et al. (1983) described an endemic Se intoxication discovered in 1961 in Enshi County, Hubei Province of China. Selenium from a stony coal entered the soil by weathering and was available from alkaline soils for uptake by crops.

In 1957, Se was identified as an essential trace element (or micronutrient) in mammals (Schwarz and Foltz 1957). Proteins containing Se were found to be essential components of certain bacterial and mammalian enzyme systems (e.g., glutathione peroxidase) (Stadtman 1974). Several Se deficiency disorders were identified, including white muscle disease in sheep and mulberry heart disease in pigs (Muth et al. 1958). In the early 1970s, Chinese researchers identified the first major human Se deficiency disease as a childhood cardiomyopathy (Keshan disease; Chinese Medical Association 1979). Thus, Se deficiency as well as toxicity can cause adverse effects in animals.

One of the most important features of Se ecotoxicology is the very narrow margin between nutritionally optimal and potentially toxic dietary exposures for vertebrate animals (Venugopal and Luckey 1978; Wilber 1980; NRC 1989; USDOI 1998). Selenium is less toxic to most plants and invertebrates than to vertebrates. Among vertebrates, reproductive toxicity is one of the most sensitive endpoints, and egg-laying vertebrates have the lowest thresholds of toxicity (USDOI 1998). The most dramatic effects of Se toxicity are extinction of local fish populations and teratogenesis in birds and fish (see Appendix A). Other effects from Se include mortality, mass wasting in adults, reduced juvenile growth, and immune suppression (Skorupa 1998).

Selenium biogeochemistry and the mechanism of entry into living cells are complex (Stadtman 1974, 1996). Se occurs in chemical forms that are analogous to forms of sulfur (S) (Sunde 1997; Fan et al. 1997, 2002; Suzuki and Ogra 2002; Kryukov et al. 2003; Moroder 2005; Unrine et al. 2007). Chief among these are elemental

Se ($Se^0$), selenide ($Se^{-2}$), selenite, and selenate, as well as methylated forms $Se_x(CH_3)_x$. Selenate and selenite can be taken up by plants and converted to organic forms. These organic forms are usually analogues to S-containing biomolecules, especially amino acids. This conversion occurs through either nonspecific isosteric substitution for S in amino acids (selenocysteine or selenomethionine), or through co-translational conjugation of selenophosphate ($SePO^{3-}$) to serine mediated by selenocysteine tRNA and selenocysteine synthase. In the latter case, selenocysteine is incorporated into genetically encoded selenoproteins (i.e., those proteins whose encoding DNA sequences have a UGA codon and a selenocysteine insertion sequence). In addition, some other metabolites, such as seleno-sugars, are known to occur.

Many enzymes and other proteins have been identified and characterized that require Se for their activity (selenoproteins). In 1973 the first functional selenoproteins were identified: glutathione peroxidase in mammals (Flohé et al. 1973; Rotruck et al. 1973) and formate dehydrogenase and glycine reductase in bacteria (Andreesen and Ljungdahl 1973; Turner and Stadtman 1973). Glutathione peroxidases are part of a large family of proteins that serve a variety of antioxidant and other functions that vary among species and specific tissues (Pappas et al. 2008). These discoveries confirmed Se as an essential nutrient and indicated a role in defense against oxidative injury. It was another decade before a second mammalian selenoprotein was identified as selenoprotein P (Motsenbocker and Tappel 1982). Selenoprotein P (SelP) is now one of the most well-documented selenoproteins. The gene sequence for SelP is highly conserved in bacteria, mammals, and fish (Tujebajeva et al. 2000). The amino acid sequence is rich in selenocysteine, histidine, and cysteine residues, suggesting a function in metal binding or chelation. In fact, SelP has been found to complex with Hg, Ag, Cd, Zn, and Ni (Yoneda and Suzuki 1997a, 1997b; Mostert et al. 1998; Sasaku and Suzuki 1998; Yan and Barrett 1998; Mostert 2000), which supports earlier reports of Se-detoxifying the effects of Hg and Cd in humans and marine mammals (Kosta et al. 1975; Hodson et al. 1984; Pelletier 1985; Osman et al. 1998).

While the glutathione peroxidases and SelP are among the best-known selenoproteins, there are many others. It is now known that the human genome contains 25 genes that encode for selenoproteins (Kryukov et al. 2003). Selenocysteine is genetically encoded by the UGA codon when it occurs with a selenocysteine insertion sequence (SECIS) in the 3′ untranslated region of the DNA sequence (Sunde 1997).

Proteins that contain selenoaminoacids that are nonspecifically incorporated into proteins during translation (i.e., not encoded by a UGA codon and a SECIS) are known as "Se-containing proteins." Selenomethionine, the Se-containing analog of methionine, can be nonspecifically incorporated into peptides because methionyl-tRNA acylase, the enzyme that charges methionyl-tRNA, does not discriminate between methionine and selenomethionine to any great extent (Moroder 2005). A few studies have suggested or demonstrated nonspecific charging of cisteinyl-tRNA with selenocysteine, which could be detrimental for proteins that require cysteine for their structure and function (Wilhelmsen et al. 1985; Müller et al. 1998; Unrine et al. 2007; Garifullina et al. 2008). Analytical identification and quantification of selenocysteine is difficult, which makes it hard to demonstrate nonspecific incorporation into proteins based on analytical data alone (Unrine et al. 2007).

## 3.3 SOURCES OF Se ENTERING AQUATIC ENVIRONMENTS

Selenium is widely distributed globally and is cycled through environmental compartments via both natural and anthropogenic processes (Nriagu 1989; Haygarth 1994). Ancient organic-rich depositional marine basins are linked to the contemporary global distribution of Se source rocks (Presser et al. 2004a). Figure 3.1 shows a global distribution of phosphate deposits (o) overlain onto that of productive petroleum (a continuum of oil, gas, and coal) basins (+) to generate a global plot of organic-carbon enriched sedimentary basins. The depositional history of these basins and the importance of paleo-latitudinal setting in influencing the composition of the deposits indicate that bioaccumulation may be the primary mechanism of Se enrichment in ancient sediments (Presser 1994; Presser et al. 2004a).

Selenium source rocks in the western United States (Figure 3.2 adapted from Seiler et al. 2003) encompass a wide range of marine sedimentary deposits, from shales mildly enriched in organic carbon to oil shales strongly enriched in organic matter, biogenic silica, phosphate, and trace elements (Presser et al. 2004a). These fine-grained sedimentary rocks provide enriched but disseminated Se sources as 1) bedrock soils for agricultural development or 2) source sediment for alluvial fans (Presser 1994). The areal extent of these rocks in the 17 western states is Upper Cretaceous, approximately 77 million hectares or 17% of the total land area, and Tertiary (mainly Eocene and Miocene), 22 million hectares or 4.6% of the total land area. Depending on their history, Tertiary continental sedimentary deposits may be seleniferous, and these deposits encompass approximately 94.7 million hectares or 20% of the total land area.

Environmental contamination by Se often is associated with particular local Se-enriched geologic formations, as, for example, the Upper Cretaceous–Paleocene Moreno and Eocene–Oligocene Kreyenhagen Formations in the Coast Ranges of California, USA (Presser 1994), the Permian Phosphoria Formation in southeast Idaho

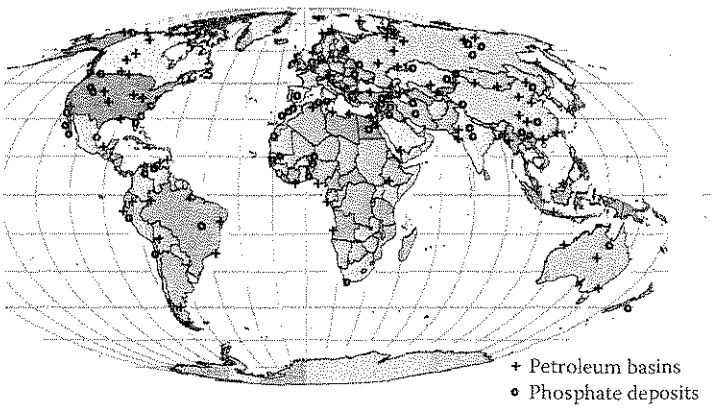


**FIGURE 3.1** Worldwide distribution of Se-rich geologic formations composed of organic-carbon enriched sedimentary basins. (Adapted from Figure 11-5 in Presser et al. 2004a; http://wwwrcamnl.wr.usgs.gov/Selenium/index.html.)

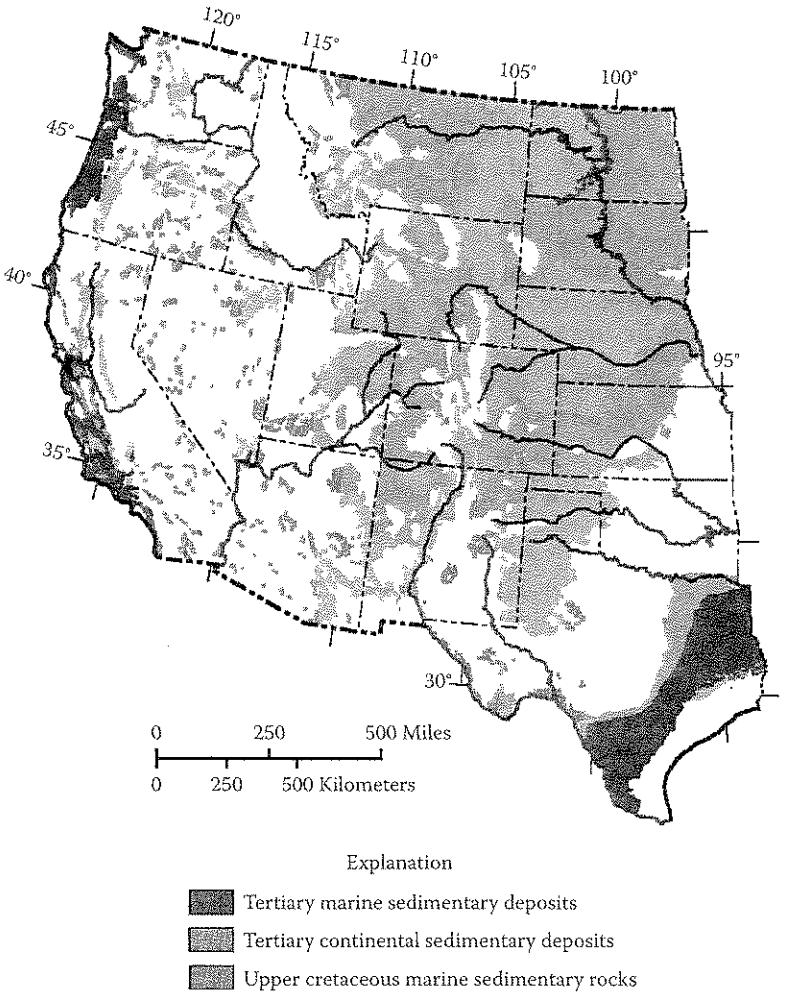


FIGURE 3.2 Selenium source rocks in the western United States. (Adapted from Seiler et al. 2003; http://pubs.usgs.gov/pp/pp1655/.)

(Presser et al. 2004b), the Cretaceous Mist Mountain Formation in Southeastern British Columbia, Canada (Lussier et al. 2003), and the Permian Maokou and Wujiaping shales in south-central China (Zhu et al. 2008). Selenium in these deposits may be present as organic and inorganic forms (Yudovich and Ketris 2006). Selenium is also associated with various sulfide ores of copper, silver, lead and mercury, and uranium (Wang et al. 1993).

Selenium is mobilized through a wide array of anthropogenic activities typically involving contact of a Se-containing matrix with water. In some cases, the contamination will be restricted to local environments, but in other instances Se can be transported a considerable distance from the place of origin.

Selenium in irrigation wastewaters is a significant environmental concern in arid and semi-arid regions (Outridge et al. 1999; Seiler et al. 2003). In areas of seleniferous soils (Figure 3.2), irrigation waters can mobilize dissolved Se predominantly in the form of selenate (Seiler et al. 2003). In these areas, drainage systems often are installed to prevent root zone waterlogging. The resulting oxic drainage water has an alkaline pH and contains elevated concentrations of salts, nitrogenous compounds, and trace elements, including Se (up to 1400 μg Se/L) (Presser and Ohlendorf 1987). Such Se-enriched drainage waters have entered aquatic ecosystems and have been associated with widespread adverse effects (Appendix A).

Although natural weathering slowly mobilizes Se from host rock sequences, this process is greatly accelerated by mining activities that expose the ore and waste rock to oxidation. Oxidized Se and associated metals can infiltrate and leach into the surrounding soils, surface water, and groundwater. Selenium release is of particular concern in coal, phosphate, and uranium mines (Ramirez and Rogers 2002; Presser et al. 2004a, 2004b; Muscatello et al. 2006). Open-pit coal (Dreher and Finkelman 1992; Lussier et al. 2003) and phosphate mines (Hamilton and Buhl 2004) are a significant source of Se because large volumes of rock overlying the target ore seams are left behind in surface waste rock dumps. Selenium is dispersed throughout these deposits but may achieve its highest concentrations in waste-shale zones that occur between the ore zones. In regions where mountaintop mining for coal is practiced, these fresh rock wastes are deposited as "valley fill," providing ideal conditions for both leaching and direct transport of Se-enriched waters into regional ponds, reservoirs, lakes, and rivers (Appendix A).

Selenium release from coal burning for power generation is a major anthropogenic source to the environment, either directly during combustion (Wen and Carignan 2007) or indirectly from disposal of solid combustion waste (coal ash) (Cherry and Guthrie 1977; Johnson 2009). Burning coal oxidizes the organic matter and creates residual wastes, both particulate "fly ash" and larger molten "bottom ash." The fly ash is of particular concern because of its high surface area–to–volume ratio, which facilitates adsorption of mobile trace elements (Jankowski et al. 2006). The resulting Se concentration in waste products may be 4 to 10 times greater than the parent feed coal (Fernández-Turiel et al. 1994). The potential ash waste volumes can be large. More than 400 coal ash disposal sites are designated in the United States. In 2007, about 131 million tons of ash waste was generated, and about 21% of this total was discharged to surface impoundments (Breen 2009). Thermal, pH, and redox conditions during coal combustion help generate predominantly selenite in the ash waste collected on electrostatic precipitators (Yan et al. 2001; Huggins et al. 2007). Selenium is readily solubilized in the alkaline conditions of aquatic fly ash settling basins or fly ash reservoirs (Wang et al. 2007). Clarified ash sluice water or sluice water return flows make their way to local receiving waters as a permitted wastewater discharge or through groundwater seepage. Selenium contamination can occur accidentally due to overfilling events or failures of containment systems. Spectacular events occur as well, such as the catastrophic December 2008 spill of 5.4 million

cubic yards of ash from a Tennessee Valley Authority coal-fired power plant (TVA 2009).

The worldwide anthropogenic Se flux to the atmosphere has been estimated at 6.4 M kg/y (Mosher and Duce 1987). Approximately 50% is from coal combustion. Smelting of non-ferrous metal ores involves intense heating to mobilize and isolate the metal of interest; the associated Se and sulfides are volatilized and released in stack gases. Up to 30% of the Se present in feed coal is emitted as a vapor phase, and about 93% of that is returned in the form of elemental Se (Andren and Klein 1975). Roughly 80% of atmospheric Se returns to the ground as wet deposition (Wen and Carignan 2007), mostly near emission sources (Wang et al. 1993). However, depending on atmospheric conditions, stack gases can be carried considerable distances. Seleniferous stack gas from a large copper smelter in Sudbury (ON, Canada) has contaminated lakes up to 30 km away (Schwarcz 1973; Nriagu and Wong 1983).

Crude oil is formed in organic carbon-enriched basins and is a source of Se to the environment. A fraction of Se in crude oil partitions to wastewaters during refining and can be discharged to the environment. Heavy crude oils produced in the San Joaquin Valley and processed at refineries that surround the northern reach of the San Francisco Bay contained 400 to 600 µg Se/L (Cutter and San Diego-McGlone 1990). The northern reach of the bay was listed as impaired by Se discharged from refineries, and control strategies were implemented to reduce Se loads to the bay in 1989 (Presser and Luoma 2006) (Appendix A).

Production and use of Se as a commodity also result in discharge of Se to aquatic systems. More than 80% of the world's production of commercially available Se is derived from anode slimes generated in the electrolytic production of copper (USGS 2000). Processing of the slimes can result in aqueous discharges of Se to surface waters (Naftz et al. 2009). Refined Se is used 1) in electronic components such as rectifiers, capacitors, and photocopy or toner products; 2) in a wide array of industrial applications, such as glass tinting, coloring of plastics, ceramics and glass; 3) as a catalyst in metal plating; and 4) in rubber production (George 2009).

Pharmaceutical applications include dietary Se supplements, anti-fungal treatments, and anti-dandruff shampoos. Each of these uses can result in Se discharges to surface waters and sewage treatment plants. Municipal landfills can generate leachates containing Se that can reach groundwater (Lemly 2004).

In some areas of the world, Se concentrations in soils are below levels adequate to produce feed and forage with sufficient Se to satisfy essential (or optimal) dietary requirements for livestock (Oldfield 1999). Selenium deficiency can be remedied by supplementing Se in feed, some of which may be excreted. Runoff from large feedlot operations where these dietary supplements are used is of particular concern because the Se is in the form of highly bioaccumulative selenomethionine (Lemly 2004). In other cases, fertilizers with nutritional Se amendments (e.g., selenate salts) are applied to lands to rectify this deficiency and enhance production (Watkinson 1983). Under some conditions, application to thin soils having low organic matter has produced short-term elevation of Se concentrations in runoff (Wang et al. 1994), which may be of concern in some receiving environments.

### 3.3.1 Future Sources of Se

Rapid progress in nanotechnology will likely benefit nearly every sector of science and industry, and consumer products containing nanomaterials are presently entering the market at the rate of 2 to 3 products per week (http://www.nanotechproject.org/). These benefits, however, come with associated risks. Selenium is a key component of nanomaterials such as CdSe or PbSe quantum dots. Quantum dots are nanometer-scale crystallites that function as semiconductors because of quantum confinement effects that occur when the size of the particles approaches the wavelength of their electrons (Reiss et al. 2009). These materials are useful in optoelectronic devices such as light-emitting diodes and photovoltaics. In addition to potential toxicity resulting from degradation of these materials and associated release of Se, emergent properties of the solid-state materials could also elicit toxic responses. For example, active electronic sites that arise from defects in crystal planes and electron hole pairs excited by ultraviolet light could lead to the generation of reactive oxygen species eliciting toxicity (Hardman 2006; Nel et al. 2006). Widespread use of Se-containing nanomaterials could lead to environmental Se contamination, and the environmental consequences may be different from those resulting from current Se sources.

### 3.3.2 Selected Se Problem Sites

Case studies documented in Appendix A represent a variety of site-specific conditions and include both freshwater and marine sites. Case studies include the following:

- Belews Lake, North Carolina, USA
- Hyco Lake, North Carolina, USA
- Martin Creek Reservoir, Texas, USA
- D-Area Power Plant, Savannah River, South Carolina, USA
- Lake Macquarie, New South Wales, Australia
- Elk River Valley, Southeast British Columbia, Canada
- Areas of the Appalachian mountains affected by mountaintop mining and valley fills
- Kesterson Reservoir, San Joaquin Valley, California, USA
- Terrestrial and aquatic habitats, San Joaquin Valley, California
- Grassland Bypass Project, San Joaquin Valley, California
- San Francisco Bay–Delta Estuary, California
- Phosphate mining in the Upper Blackfoot River watershed, Idaho

Each study compiles information on sources, fate and transformation, effects, and lessons learned. Each case study is distinct with respect to biological receptors; attributes of water, sediment, particulates; food-web pathways; community complexity; and the extent of bioaccumulation and observed effects. A synopsis of 12 case studies, representing diverse Se sources, was previously provided by Skorupa (1998).

A variety of Se contamination events have occurred over the past 40 years in aquatic systems. There have been a number of investigated case studies where elevated environmental Se was attributed to disposal of power plant coal-combustion wastes. These

cases (Appendix A) include situations where fly ash was released directly into a nearby water body (e.g., D-Area power plant at Savannah River) or more commonly held in ash settling ponds and the pond effluent released into lakes or reservoirs (e.g., Belews and Hyco Lakes, Martin Reservoir, and Lake Macquarie). In particular, the Belews and Hyco Lakes case studies provided some of the earliest and best-documented evidence of Se effects in the aqueous environment. In some of these cases, confounding factors such as release of other co-occurring contaminants or lack of sufficient information about ecosystem conditions prior to Se addition have made it difficult to ascribe adverse impacts specifically to Se, even though Se toxicosis is well established.

In the now classic study of Belews and Hyco Reservoirs in North Carolina, fly ash pond effluents containing high concentrations of Se were released into the reservoirs for a decade. Both reservoirs experienced reproductive failure of fish populations, transforming formerly diverse fish communities to communities dominated by a few Se-insensitive fish species (Cumbie and Van Horn 1978; Lemly 2002). Fly ash wastewater discharges were later curtailed and a diverse fish community, including Se-sensitive species, was re-established in both waterbodies within several years. However, at each location, more than 20 years later, Se bioaccumulation remains elevated relative to reference sites.

The most well-known case of Se bird poisoning in a field environment is the impoundment of Se-enriched agricultural drainage water in Kesterson Reservoir in the San Joaquin Valley of California. High levels of dissolved Se in drainwater were taken up into the food web, affecting aquatic-dependent wildlife (birds) that showed signs of Se poisoning in adults, as well as reproductive failure due to embryo teratogenesis and failure to hatch (Ohlendorf et al. 1986; Presser 1994). Inputs of irrigation drainwater were halted in the late 1980s, and the reservoir was filled and capped to reduce contact of water with Se-contaminated sediments. Monitoring of ephemeral ponds in the Kesterson area since then shows Se concentrations ranging from 15 to 247 µg Se/L. Aquatic invertebrates collected from these ponds have Se body burdens ranging from 8 to 190 mg/kg dry weight (dw), but Se-induced toxicity has not been observed in aquatic birds (Skorupa 1998). After the capping of Kesterson Reservoir, additional sites receiving agricultural irrigation water were assessed (see case studies in California, Appendix A).

Following the findings at Kesterson Reservoir, the United States Department of the Interior (USDOI) in 1985 initiated the National Irrigation Water Quality Program. Reconnaissance monitoring, or field-level screening, took place at 39 areas in the western United States, where wildlife populations were considered potentially at risk due to agricultural irrigation practices in areas of known seleniferous geological deposits (Presser et al. 1994; Seiler et al. 2003). By 1993, results had confirmed that Se was the contaminant of primary concern at the National Irrigation Water Quality Program study sites, and the receptors generally at greatest risk were water birds (Seiler et al. 2003). Seiler et al. (2003) identified the following sites for further study or remediation planning because these areas were classified as embryotoxic on the basis of Se concentrations in bird eggs:

- Tulare Basin, San Joaquin Valley, California
- Salton Sea, California

- Middle Green River Basin, Utah
- Stillwater Management Area, Nevada
- Kendrick Reclamation Project, Wyoming
- Gunnison-Grand Valley Project, Colorado
- San Juan River area, New Mexico
- Sun River area, Montana
- Riverton Reclamation Project, Wyoming
- Belle Fourche Reclamation, South Dakota
- Dolores-Ute Mountain Area, Colorado
- Lower Colorado River Valley, Texas
- Middle Arkansas Basin, Colorado-Kansas
- Pine River area, Colorado

In some cases, a combination of Se sources has been identified as contributing to elevated levels of Se in ecosystems. For example, the San Francisco Bay–Delta Estuary case study addresses both agricultural drainage-driven inputs plus industrial wastewater contributions. In such instances, an accurate picture of the relative contribution of the multiple sources (e.g., independent characterization of source Se loading and speciation) is useful to conceptually or mechanistically model the ecosystem.

Studies demonstrating the growing potential of Se-related impacts relating to mining activities include coal mining and phosphate mining (Appendix A). Open pit mining practices have in the past produced "pit lakes" with elevated Se concentrations when mining activities were terminated. Mining in areas with productive coal bed or ore deposits results in the weathering of Se from mining overburden material and, in some areas, contamination of groundwater that subsequently seeps into surface water areas. In the Elk River Valley of southeastern British Columbia, open coal pit mining over the past decades has resulted in sharply increasing surface water Se concentrations. Selenium concentrations in discharges (primarily selenate) often exceed 300 µg/L. Downstream of the mines, lotic, lentic, and marsh areas are receiving substantial Se loads, leading to bioaccumulation in macrophytes, benthic macroinvertebrates, and a variety of secondary consumers. Individual-level early life stage effects have been observed in 2 fish species, marsh and water birds, and frogs, but population-level effects linked to Se have been more difficult to establish in field studies (Harding et al. 2005; Orr et al. 2006; Canton et al. 2008).

## 3.4 SELENIUM CYCLING AND BIOACCUMULATION IN AQUATIC ECOSYSTEMS

Figure 3.3 is a conceptual model of Se dynamics and transfer in aquatic ecosystems. The model illustrates the steps that determine Se effects in ecosystems. Those steps are described in detail below.

### 3.4.1 Selenium Speciation in Water, Particulates, and Biota

Selenium from natural and anthropogenic sources typically enters aquatic ecosystems as the oxidized inorganic anions, selenate ($Se^{+4}$) and selenite ($Se^{+6}$), although

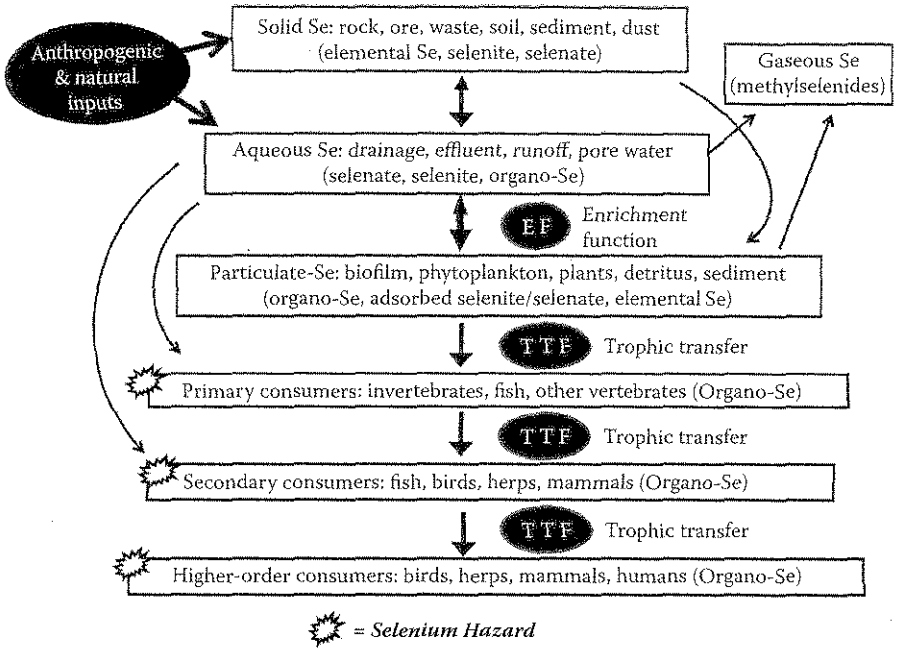


FIGURE 3.3 Conceptual model of Se dynamics and transfer in aquatic ecosystems.

small amounts of dissolved organic Se compounds ($Se^{-2}$) also can be present in water due to biological activity. Selenate and selenite can be the predominant species present in the water columns of aquatic ecosystems (Figure 3.3). While the aqueous phase is operationally defined as materials passing through a filter with 0.45 μm or smaller pore diameter, colloidal (non-dissolved) Se may be present in this fraction. In terms of mass balance, transport of Se via sediment is usually a lesser route of entry for Se into aquatic ecosystems. However, in terms of biological reactivity, suspended material in an ecosystem plays an important role determining the effects of Se.

The biogeochemical cycling of Se in aquatic systems is characterized by the predominance of biologically mediated reactions over thermodynamically driven reactions (Stadtman 1974, 1996; Oremland et al. 1989, 1990). Both selenate and selenite anions can be actively taken up by microbes, algae, and plants and converted to organic Se compounds, including Se analogues of sulfur-containing biomolecules (Fan et al. 1997, 2002; Stadlober et al. 2001). Selenium is sequentially reduced to $Se^{-2}$ before it is ultimately incorporated into the amino acids selenocysteine and selenomethionine (Sunde 1997). Selenomethionine is the primary organic form of Se at the base of aquatic food webs. Selenocysteine is primarily present in selenoproteins in which the selenocysteine is genetically encoded. Selenocysteine is readily oxidized, indicating that it should not be persistent under ambient conditions outside of organisms. Selenocysteine typically accounts for a relatively small proportion of total Se in most plants with elevated Se concentrations, where excess Se accumulates as selenomethionine (Wu 1998). For these reasons, selenomethionine is thought to

be the primary organic form of Se relevant to bioaccumulation and toxicity in food webs (Fan et al. 2002).

For example, Se often enters a stream as selenate. If that stream flows into a wetland and is retained there with sufficient residence time, then recycling of Se may occur. During recycling, particulate Se is generated from dissolved Se species. The transformed reduced species are then returned to the water as these organisms die and decay. The more recycling, the more organo-Se and selenite are produced. Neither of these latter forms can be easily reoxidized to selenate because that reaction takes hundreds of years (Cutter and Bruland 1984). The net outcome of recycling in a watershed is a gradual build-up of selenite and organo-Se in the system. Thus, biologically mediated reactions drive conversions among dissolved species and transformation of dissolved Se to particulate species.

Bacterially mediated reactions can also produce volatile methylated Se species, which are rapidly lost to the atmosphere, or insoluble elemental Se(0), which tends to accumulate in anaerobic sediments (Fan et al. 1998; Turner et al. 1998; Peters et al. 1999; de Souza et al. 2001).

### 3.4.2 Selenium Uptake and Transfer in Aquatic Food Webs

Fine particulate organic matter, composed of living and dead biotic material and some associated inorganic particles, may contain varying proportions of inorganic and organic Se species. Consumption of these particles by primary consumers, typically invertebrates and small fish, is the primary pathway for Se entry into aquatic food webs (Figure 3.3).

Partitioning between water and particulates is a dynamic biogeochemical process that is difficult to model because equilibrium geochemical modeling fails to describe major biological processes. However, Se partitioning for any location and time can be described by a distribution coefficient or enrichment function (EF), which describes the relationship between Se concentrations in particulate and dissolved phases:

$$\text{EF} = \text{Se concentrations in particulates (micrograms/kg dw)/Se concentrations in water (}\mu\text{g/L)} \quad (1)$$

The EF usually refers to a simple ratio, as described here, but can be elaborated into a more complex enrichment function that describes variation in Se uptake in response to different environmental factors. Presser and Luoma (2010) compiled data from 52 field studies in which both water-column and particulate Se concentrations were determined. They calculated EFs, which they termed the partitioning coefficient, $K_d$. The $K_d$s across the variety of ecosystems (ponds, rivers, estuaries) vary by as much as 2 orders of magnitude (100 to 10,000) and measure up to 40,000. Most rivers and creeks show $K_d$s of >100 and <300 (e.g., San Joaquin River [CA, USA] at 150). Lakes and reservoirs usually have $K_d$s > 300, with many in the 500 to 3,000 range (e.g., Belews Lake [NC, USA] at 3,000). Those $K_d$s >3,000 are usually associated with estuary and ocean conditions (e.g., San Francisco Bay [CA, USA] at 10,000 to 40,000). Exceptions from this categorization can occur as a result of speciation effects and other site-specific conditions.

The EF represents the outcome of Se transformations occurring in a specific ecosystem, but it does not differentiate those processes. There have been few attempts to develop biogeochemical models to quantify these processes (Meseck and Cutter 2006). For ecosystem-scale modeling, EF is estimated from field determinations of dissolved Se concentrations and Se concentrations in one or more types of particles. It is recognized that this operational EF will vary widely among environments. An important part of the methodology is to use the characteristics of the environment in question to narrow the potential variability. Hence, it is critical for site-specific Se assessments to quantify Se concentrations in particulates forming the base of the food web.

Bioaccumulation of Se from particulates by primary aquatic consumers is a key determinant of dietary Se exposure and, therefore, of the risk of Se toxicity to higher-order aquatic consumers (e.g., predatory fish and aquatic birds) (Figure 3.3; Wang 2002; Luoma and Rainbow 2005, 2008). Biodynamic models, which characterize the balance between gross Se influx rate and the gross efflux rate, can be the basis for modeling Se bioaccumulation and trophic transfer in aquatic ecosystems (Presser and Luoma 2010). For primary consumers, biodynamic experiments indicate that uptake of dissolved Se is negligible compared with Se uptake from diets of fine particulates (Luoma et al. 1992). With simplifying assumptions (i.e., no uptake of dissolved Se and no growth), the exposure equation for consumers is

$$C_{\text{consumer}} = [(\text{AE})(\text{IR})(C_{\text{diet}})]/[k_e] \quad (2)$$

The species-specific information in this equation (ingestion rate [IR], assimilation efficiency [AE], and efflux rate constant [$k_e$]) can be determined from kinetic experiments with invertebrates that serve as the basis of many important food webs (see Chapter 5). These parameters can be combined to calculate a trophic transfer factor (TTF) for Se. The modeled TTF characterizes the potential for a consumer to bioaccumulate Se from its diet, based on the balance of Se influx and efflux. Because TTF is defined as the Se concentration in a consumer (mg/kg dw) divided by Se concentration in diet (mg/kg dw), the preceding equation can be expressed as

$$\text{TTF} = (\text{AE})\ (\text{IR})/k_e \quad (3)$$

Selenium TTFs determined for marine and freshwater invertebrates vary widely, from 0.6 for amphipods to 23 for barnacles (Presser and Luoma 2010; Chapter 5). This variation in TTF is propagated by trophic transfer, making some food webs and some predatory taxa more vulnerable to Se bioaccumulation and toxic effects.

Biodynamic models have been developed primarily for invertebrates feeding on particulate organic matter, but the same modeling approach can also be applied to higher-order consumers, such as fish feeding on invertebrates or other fish (Baines et al. 2002). Selenium TTFs for predatory fish are less variable (range, 0.6 to 1.7; mean 1.1) than those for invertebrates (Presser and Luoma 2010). The conceptual model (Figure 3.3) summarizes Se transfer from water to organic particulates at the base of the food web to primary consumers and predators. Food web modeling based on EFs and TTFs is illustrated in more detail by Presser and Luoma (2010) and in Chapter 5.

### 3.4.3 Food-Web Exposure and Toxicity Risks

Biodynamic modeling can provide insight into variability of Se exposures among different ecosystems and different trophic levels. Selenium TTFs are useful metrics for understanding this process because they describe the bioaccumulation in animals across each trophic linkage. Contaminants that biomagnify would be expected to have TTFs >1.0 at each trophic linkage within a food chain. Although Se TTFs are variable among different ecosystems, they tend to be similar within groups of related species or species with similar trophic status. It is clear that the majority of food chain enrichment with Se occurs at the lower trophic levels and that less enrichment occurs at higher trophic levels. A compilation of TTFs for Se indicates that, for freshwater primary consumers, TTFs range from 0.9 for amphipods to 7.4 for zebra mussels; TTFs for fish average 1.1 (Presser and Luoma 2010). These observations have important implications for problem formulation and risk assessment. Unlike contaminants that strongly biomagnify in higher trophic levels (e.g., DDT and Hg), for Se, secondary and tertiary consumers may not experience substantially higher Se exposure than lower trophic levels, because enrichment of Se in aquatic food webs primarily occurs in particulates and primary consumers. For example, a recent study suggests that amphibian larvae that primarily graze periphyton actually bioaccumulate higher Se concentrations than do predatory fish in the same system (Unrine et al. 2007).

However, to establish risk, Se exposure and the magnitude of Se bioaccumulation must be considered along with an animal's sensitivity to Se. Birds and fish (predators) are the 2 taxa of animals most sensitive to aquatic Se contamination (i.e, they are the first to express the effects of Se within ecosystems), with embryonic and larval life-stages being of particular concern. Invertebrates, on the other hand, are relatively insensitive to Se (Lemly 1993a; Presser and Luoma 2006). Thus, the organisms that are most at risk are higher-order predators.

Risks of toxicity to aquatic organisms may be driven by differences in Se exposure mediated by food-web transfer. In a toxicological sense, Se sensitivity is an inherent property of the species. However, differences in Se exposure among ecosystems may be more significant than differences in the toxicological sensitivity among species. Trophic structure (who is eating whom) is as important as trophic position (food chain length) in determining Se bioaccumulation within food webs (Stewart et al. 2004; Presser and Luoma 2010). Combining site-specific estimates of EFs with generic TTFs for different taxonomic groups or species of invertebrates, fish, and birds can help explain how environmental Se concentrations will differ among ecosystems that exhibit differing ecological and biogeochemical characteristics.

## 3.5 ADVERSE EFFECTS OF Se

Risk assessment protocols for most contaminants consider 2 thresholds: 1) concentrations that cause adverse effects following short-term exposures (acute toxicity) and 2) concentrations that cause adverse effects following long-term exposure (chronic effects). Because adverse effects due to Se exposure are predominantly related to food web exposure, the standard concept of acute Se toxicity based on aqueous exposures has limited applicability in nature.

Chronic dietary toxicity from Se is manifested primarily as reproductive impairment due to the maternal transfer of Se, leading to embryotoxicity and teratogenicity (Gillespie and Bauman 1986; Lemly 1993b, 1998; Skorupa 1998; Ohlendorf 2003). This is particularly true for egg-laying vertebrates because Se is incorporated into egg yolk proteins (Kroll and Doroshov 1991; Davis and Fear 1996; Unrine et al. 2006). In addition to reproductive impairment, Se has a variety of other sublethal effects, including reductions in growth and condition index (Sorenson et al. 1984; Heinz et al. 1987; Ohlendorf 2003), tissue pathology (Sorenson et al. 1982a, 1982b, 1983a, 1983b, 1984; Sorenson 1988), and induction of oxidative stress (Spallholz and Hoffman 2002; Palace et al. 2004). Selenium can be lethal to adult organisms (Ohlendorf 1989, 2003; Heinz 1996) as demonstrated by mass mortalities of adult coots (*Fulica americana*) that occurred in agricultural drainwater habitats in California (USA) (Skorupa 1998). However, most aqueous and dietary concentrations of Se encountered by wildlife are not high enough to be lethal to adults.

Chronic toxicity to birds and fish is strongly associated with concentrations of the Se-substituted amino acid, selenomethionine, in diets and tissues of exposed biota. Studies with mallards (*Anas platyrhynchos*) have demonstrated that diets containing the naturally occurring form of selenomethionine (L-selenomethionine) were more toxic than diets containing either the synthetic enantiomeric mixture, D,L-selenomethionine, or inorganic Se (as selenite) (Heinz et al. 1988; Hoffman et al. 1996). Hamilton et al. (1990) demonstrated that toxic effects of artificial diets spiked with selenomethionine fed to Chinook salmon (*Onchorhynchus tshawytscha*) were similar to effects of diets prepared from wild mosquitofish (*Gambusia affinis*) collected from Se-contaminated habitats.

The sensitivity of aquatic taxa to Se toxicity, expressed in relation to Se concentrations in tissues or diets, varies widely among fish and aquatic-dependent birds (Staub et al. 2004). Concentrations of Se that cause adverse effects may differ substantially even between closely related species, such as rainbow trout (*Onchorhynchus mykiss*) and cutthroat trout (*O. clarki*; see Chapter 6). Similarly, 2 species of wading birds in the family Recurvirostridae showed widely differing effect concentrations for embryo hatchability and teratogenicity, with the black-necked stilt (*Himantopus mexicanus*) being much more sensitive than the American avocet (*Recurvirostra americana*) (Skorupa 1998).

The effects of Se on the survival and reproduction of individuals can lead to adverse changes to populations and community structure (Figure 3.4) (Garrett and Inman 1984; Lemly 1993a). Population and community-level effects have been primarily documented in aquatic systems where movement of organisms (emigration and immigration) is restricted. In the classic example of Belews Lake (NC, USA), 26 of 29 resident fish species experienced local extinction (Appendix A) due to reproductive failure caused by Se (Lemly 1993b, 1998).

Elimination of species from communities, particularly those taxa that exert strong top–down (some predators) or bottom–up (some microbes or benthic invertebrates) effects may have ecosystem-wide repercussions, particularly when sufficient functional redundancy is absent in the system. Se-induced shifts in community composition due to declines of certain invertebrate or forage fish species could result in reduced quality and/or quantity of food resources for higher trophic-level consumers.

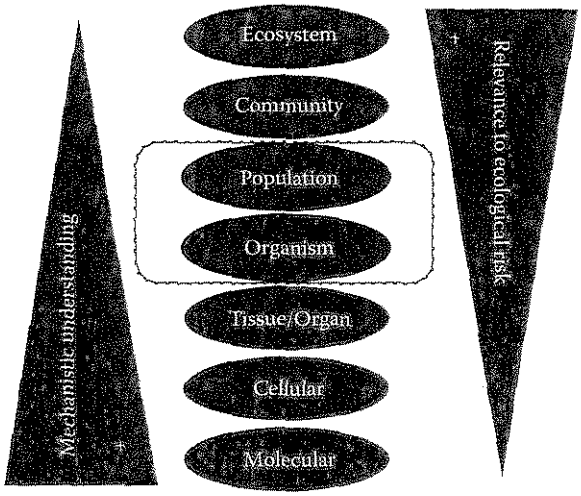


**FIGURE 3.4** Hierarchy of effects across levels of biological organization.

Most of what we know about Se bioaccumulation and toxicity comes from studies of birds and fish, but relatively little is known about Se toxicity in other vertebrates. The process of maternal transfer of Se in viviparous vertebrates (i.e., mammals and some herpetofauna) is poorly understood, but it appears that the margin between essentiality and toxicity of Se is much broader for placental mammals than for egg-layers (NRC 1980; see Chapter 6). Thus, among vertebrates, the most notable knowledge gap regarding Se exposure and toxicity is for oviparous species of amphibians and reptiles. This knowledge gap prevents phylogenetic comparisons regarding Se sensitivity.

Amphibians and reptiles are among the most critically endangered vertebrates (Gibbons et al. 2000; Stuart et al. 2004; Wake and Vredenburg 2008). Collectively referred to as "herpetofauna," they are also ecologically important in both aquatic and terrestrial ecosystems. As ectotherms with low energy requirements, herpetofauna can achieve high biomasses compared with mammals and birds occupying similar trophic levels (Hopkins 2006, 2007). In numerous ecosystems, where vertebrate numbers and biomass have been carefully calculated, salamanders, frogs, lizards, and snakes have been shown to be far more abundant than most other vertebrates (Burton and Likens 1975; Roughgarden 1995; Rodda et al. 1999; Petranka and Murray 2001; Gibbons et al. 2006). Thus, herpetofauna greatly influence the cycling of energy and nutrients in many ecological systems (Seale 1980; Wyman 1998; Bouchard and Bjorndal 2000; Beard et al. 2002; Ranvestel et al. 2004; Gibbons et al. 2006; Regester et al. 2006) and may play significant roles in the cycling of contaminants, including Se, in food webs (Hopkins 2006, 2007; Hopkins and Rowe 2010).

In a system contaminated with coal combustion wastes in South Carolina (USA), water snakes (*Nerodia fasciata*) accumulated elevated concentrations of Se from the fish and amphibians they ingested (Hopkins et al. 1999). Based on indirect evidence from long-term controlled feeding studies (Hopkins et al. 2001, 2002a) and additional field studies on amphibians (Roe et al. 2005; Hopkins et al. 2006), it appears

that the elevated Se concentrations in snakes were more likely due to ingestion of amphibians than fish (Hopkins 2006). No studies have evaluated the importance of amphibian and reptilian prey as pathways of Se exposure to fish, birds, or mammals that commonly ingest them. Nor have any studies rigorously examined bioaccumulation and effects of Se in top trophic-level reptiles such as snapping turtles and alligators, despite many traits that make these species desirable for ecotoxicological studies (Hopkins 2000, 2006; Roe et al. 2004; Bergeron et al. 2007).

Like birds and fish, reptiles and amphibians partition significant quantities of the Se they accumulate into their ovaries, with subsequent maternal transfer to their eggs. Turtles, alligators, snakes, lizards, and frogs have all been shown to maternally transfer Se (Nagle et al. 2001; Hopkins et al. 2004a, 2005a, 2005b, 2006; Roe et al. 2004). In controlled feeding studies with lizards and field surveys of frogs, 33% to 53% of a female's total body burden of Se prior to oviposition was transferred to her follicles or eggs (Hopkins et al. 2005a, 2005b, 2006). Spinal deformities in Columbia spotted frog embryos with Se concentrations up to 20 mg/kg dw were documented in the Elk River Valley (BC, Canada) watershed (Appendix A).

The reproductive effects and developmental consequences of Se deposition into reptilian eggs remain largely unexplored. A field study with adult amphibians demonstrated that females that transferred excessive concentrations of Se and Sr to their eggs also experienced significant reproductive impairment, including teratogenic effects characteristic of Se toxicity (Hopkins et al. 2006; discussed in more detail in Chapter 6). Additional field studies and controlled dietary exposures linked to adverse reproductive outcomes, much like those conducted on birds and fish, are needed for these diverse and threatened group of vertebrates.

## 3.6 ECOSYSTEM RECOVERY FOLLOWING Se CONTAMINATION

A limited number of examples are available which document the recovery of impacted aquatic populations in Se-contaminated ecosystems. The recovery of the warm water fish community in Belews Lake represents the most comprehensive example currently available. Prior to being impacted by coal ash effluent, the Belews Lake fish community was diverse, with 29 species. The lake began receiving Se-laden ash pond effluents in 1975. The changes in the warmwater fish community in Belews Lake were documented by sampling lake coves during the period 1977 to 1984, coupled with muscle tissue Se measurements in selected taxa collected from trap nets or by electrofishing (Barwick and Harrell 1997). Monitoring showed significantly reduced fish diversity and biomass during 1977 to 1981, as the lake continued to receive some Se-laden ash pond effluents. In 1978 only 7 taxa were present; in 1979 only 3 were collected. By the mid-1980s, all seleniferous loading to the lake from ash pond effluent was curtailed. Fishery monitoring in successive years indicated a gradual reestablishment of a diverse community, as the range of species successfully expanded downlake from a relatively unimpacted headwater area (Lemly 1997; Barwick and Harrell 1997). By 1985, as median Belews Lake Se water column concentrations decreased to <5 µg/L, 21 fish species had returned to the main body of Belews Lake (1984 and 1985 data; Barwick and Harrell 1997). By 1990, within

5 years of termination of ash pond effluents, 26 fish taxa (combined 1984–1990 data) had been documented (Barwick and Harrell 1997).

Compared with these population-level responses that indicated recovery of the system over a 5-year period, Se residues in monitored taxa, including catfish (*Ameiurus* spp. and *Ictalurus* spp.), green sunfish (*Lepomis cyanellus*), and bluegills (*L. macrochirus*), were slow to decrease. Muscle Se concentrations in these taxa decreased from average concentrations (converted to dry weight from wet weight, using an estimated 75% moisture content) of 42 mg/kg in catfish and 87 mg/kg in green sunfish during 1983–1987, to levels between 4.0 and 15 mg/kg, respectively, by 1992. Those concentrations remained well above reference-site fish residues, however, and low frequencies (up to 6%) of malformed fish larvae continued to be reported as late as 1996 (Lemly 1997). A continuing decline in fish Se concentrations has been closely linked with gradually declining Se concentrations in sediment and benthic food webs in Belews Lake.

Following the termination of drainwater inputs and the filling of the ponds at Kesterson National Wildlife Refuge, monitoring and modeling indicated that reduced, but persistent, Se exposures from the terrestrial habitat and ephemeral pools would continue to present a low level of risk to wildlife (Ohlendorf 2002). Although Se concentrations in specific food webs remained above toxicity levels of concern and slightly elevated with respect to reference sites, Ohlendorf (2002) concluded that Se concentrations in terrestrial and aquatic wildlife did not pose substantial risk of adverse effects on reproductive or other responses.

Under some conditions, recovery of populations of a specific receptor species may not take place. For example, Se amendments made to a series of Swedish lakes with elevated levels of mercury in the 1980s is thought to have resulted in the local extirpation of perch (*Perca fluviatilis*) from several lakes isolated from source populations (Paulsson and Lundbergh 1989; Skorupa 1998).

In summary, these cases indicate that some aquatic populations may recover in the several years following the cessation of aqueous Se inputs. However, aquatic communities commonly include important benthic food webs. Selenium concentrations in sediment typically decline more slowly than water column concentrations. Therefore, natural attenuation of Se in food webs may require several years or even decades.

## 3.7 STRATEGIES FOR ASSESSING THE RESOURCE TO BE PROTECTED

### 3.7.1 System Characteristics

Source, habitat, and food web characteristics, along with other stressors, influence Se's overall effect on an ecosystem (Figure 3.5). These characteristics are important in developing a strategy to assess an ecosystem that may be at risk from Se contamination.

Both the amount and the chemical form of Se discharged into an ecosystem help to determine its fate and effects. Most often, Se enters aquatic systems as a highly water-soluble oxyanion (i.e., selenate or selenite). In typical coal combustion wastewaters, for example, most of the Se enters the ecosystem as selenite. The efficiency of uptake

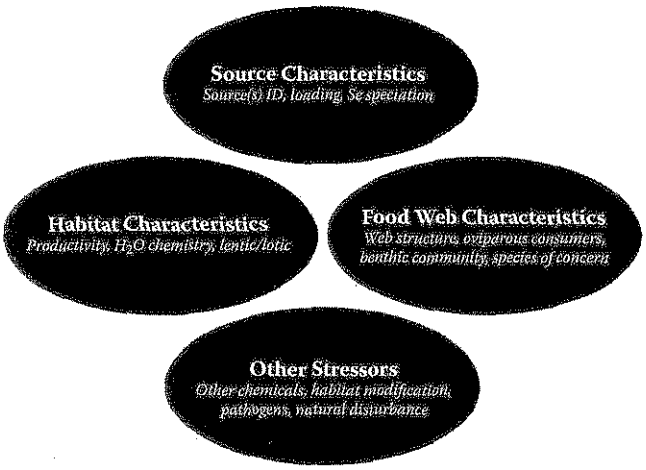


FIGURE 3.5 Ecosystem characteristics that influence Se cycling, bioavailability, and effects.

by plankton from the water column is greater for selenite than for selenate, resulting in a rapid flux of Se into the aquatic food web (Besser et al. 1989, 1993; Riedel et al. 1996). Relative to selenate, selenite is also more readily complexed and precipitated from the water column via non-biological pathways (e.g., by co-precipitation with metal hydroxides: NAS 1976; Simmons and Wallschläger 2005). These properties tend to favor incorporation of selenite-Se into particulates, which facilitates a benthic exposure pathway for consumers. Increased severity and rate of manifestations of selenite-Se-induced toxicity observed in biota (e.g., at Belews Lake), relative to ecosystems receiving a similar or greater concentrations of selenate-Se (e.g., Kesterson Reservoir), have been attributed to these differences in source speciation (Skorupa 1998; Appendix A). Source characterization should include temporal analyses both as a means to accurately assess loading rates and to confirm Se speciation over time.

The conditions within a receiving water body are important factors contributing to Se accumulation within components of food webs. The most severe Se toxicity problems documented to date have occurred in lentic systems with elevated Se inputs and comparatively long residence time. High biological productivity tends to increase the rate of incorporation of dissolved inorganic Se into biota, resulting in high concentrations of bioavailable Se in biota and organic detritus (Orr et al. 2006). Systems with lower productivity and shorter residence times result in less accumulation of Se. High levels of microbial activity are typical of high-productivity lentic and wetland habitats that are most often also associated with high levels of Se bioavailability. This is not surprising, because microbially mediated reactions are involved in many of the transformations that affect Se fate and bioavailability, including reduction of selenate (least bioavailable) to selenite (more bioavailable) and reduction of these inorganic species to organic selenides (most bioavailable) (Riedel et al. 1996). Microbial activity can also lead to reduced Se bioavailability, for example, by formation of elemental Se, an insoluble form that tends to accumulate in sediments, or loss from the aquatic system by formation of volatile methylselenide species (Fan et al. 1998).

In aquatic ecosystems with low productivity and short residence times the accumulation of Se in the food web is expected to be reduced. For example, Adams et al. (2000) demonstrated significant differences in the accumulation of Se in fish from lotic versus lentic systems. However, the fate of Se in some localized habitats may vary widely. In sites such as marginal wetlands, side channels, and seasonally flooded areas, local hydraulic residence time is longer and productivity is higher than in main-channel habitats. This leads to greater Se accumulation in organic detritus and organic-rich sediments, greater biotransformation of inorganic Se, and greater Se bioaccumulation.

Hydrology, productivity, and microbial activity of aquatic habitats influence the quantity and type of fine particulate organic matter available at the base of aquatic food webs. These differences are reflected in the speciation and bioavailability of particulate Se (Presser and Luoma 2010). Operationally defined EFs characterize Se partitioning between water and particulate matter for aquatic systems. Systems with relatively low EFs (<500) are streams, whereas systems with the highest EFs (>2000) tend to be dominated by highly productive wetlands and estuaries.

The magnitude of EFs for primary producers is an important determinant of the potential for Se bioaccumulation in food webs. The fate of Se entering aquatic food webs, however, is further modified by differences in food web structure among aquatic ecosystems. The Se exposure of higher-order predators is predominantly determined by the specific taxa that comprise these links rather than the number of trophic links in their food webs. Predators that consume aquatic taxa such as marine bivalves, which have exceptionally high TTFs (range: 1.4 to 23), may experience greater Se exposure than other predators in the same ecosystems (Presser and Luoma 2010).

Food web linkages to the top oviparous consumers should be included in Se site assessments. Reproductive impairment and early life stage malformations in high trophic-level egg-laying (oviparous) vertebrate species, including fish and aquatic-dependent birds, are the most frequently documented manifestations of Se toxicity. Understudied oviparous species, such as reptiles and amphibians, can make up a substantial fraction of biomass and are critical components in system energy transfer and ecology.

Food web structures and hence the potential for dietary Se exposure of top predator species are commonly highly complex. Consumers utilize a wide variety of food sources that are influenced by season, migratory patterns, or life stage–dependent factors. Temperate lentic habitats, when provided with sufficient soluble nutrients, support a robust but seasonally variable food web. Partitioning of water-column Se in particulates is efficient, as reflected in higher EFs in lentic versus lotic systems. Benthic organisms comprise an important component of both lentic and lotic food webs, but lentic sediment is typically comprises fine particulates, including biogenic particulate organic material. The organic component (total organic carbon) of sediments has been associated with higher Se concentrations and, further, strongly appears to influence the magnitude of Se bioaccumulation in benthic invertebrates. In lotic systems, substrates and stream velocities are less amenable to accumulation in fine particulate material and detritus, except in backwater areas, which are essentially lentic habitats.

The length of the food web and number of trophic levels represented may not reflect the magnitude of the risks posed by environmental Se contamination to species of concern. In San Francisco Bay (Appendix A), white sturgeon (*Acipenser transmontanus*), an exceptionally long-lived top predator, consume great quantities of an invasive clam species (*Potamocorbula amurensis*). While Se concentrations remain relatively low in both the water column (<1 µg/L) and suspended particulates (0.5 to 1.5 mg/kg dw), Se is bioaccumulated efficiently to potentially problematic Se concentrations by sturgeon because of the approximate 6-fold trophic transfer from particulate to clam. In the same ecosystem, juvenile striped bass (*Morone saxatilis*) utilize a slightly longer food web comprising first- and second-order crustacean consumers (zooplankton and mysid shrimp). The bass do not accumulate Se to problematic concentrations because trophic transfer is less than 2-fold. In mechanistic terms, the key difference between the 2 food webs, and therefore the exposures of predatory fish, is the very low efflux rate of Se from clam tissue relative to the crustacean food items (Stewart et al. 2004).

Se toxicity may be enhanced by other ecological variables normally encountered by animals in nature. For example, Chapter 6 discusses potential temperature effects on Se toxicity (e.g., the Lemly [1993c] "Winter Stress Syndrome"). Selenium-induced shifts in community composition due to declines of certain invertebrate or forage fish species could result in reduced quality and/or quantity of food resources for higher trophic-level consumers. Such indirect effects mediated through nutritional deficits are widespread in systems contaminated by other contaminants (Fleeger et al. 2003), including complex waste mixtures containing Se (Hopkins et al. 2002b, 2004b; Roe et al. 2006). Possible interactions between Se and abiotic variables (e.g., temperature, salinity, climate), life history events (e.g., migration, metamorphosis), and other anthropogenic factors (e.g., eutrophication, habitat modification, interactions with other contaminants) are also knowledge gaps that need to be addressed to better inform future risk of Se to aquatic biota.

Another major challenge to evaluating Se toxicity is its well-documented interaction with other constituents of aquatic environments. For example, sulfate inhibits uptake of selenate by plants and has an antagonistic effect on the acute toxicity of selenate (dissolved route of exposure only) to invertebrates and fish (Brix et al. 2001). However, sulfate–selenate interactions have not been shown to influence Se transfer via trophic transfer, which is the primary exposure mechanism for chronic toxicity (Besser et al. 1989; Skorupa 1998; Presser and Luoma 2010). A more significant challenge to evaluating Se toxicity in the field is its common co-occurrence with other contaminants. Many of the industrial sources of Se also emit additional trace elements and in some cases organic contaminants. For example, coal combustion produces solid waste containing elevated concentrations of more than a dozen potentially toxic trace elements (Rowe et al. 2002). This complication is not unique to Se, because all habitats on the planet contain measurable concentrations of other contaminants. However, for Se this may become a major source of uncertainty because it is well known that Se interacts with other contaminants such as Hg and As (Cuvin-Aralar and Furness 1991; Yoneda and Suzuki 1997a, 1997b; Heinz and Hoffman 1998; Hopkins et al. 2006, 2007). Synergistic, additive, and/or antagonistic interactions are likely in some Se-contaminated systems. These interactions are complex

and are likely to be site specific. Revealing the molecular mechanisms behind these interactions with Se also may allow better predictive power in these situations.

Investigation of population-, community-, and ecosystem-level responses to Se contamination also may be complicated by the presence of other stressors such as habitat modification, altered hydrology, species introductions, diseases, and the like. Each of these factors would be relevant for establishing hypothetical or actual reference site conditions, as would consideration of natural successional stages.

### 3.7.2 Assessment Endpoints and Measures of Exposure and Effect

When episodes of Se contamination occur or are suspected, it is useful to have a method to assess the possible adverse effects on the ecological systems in the field. The Ecological Risk Assessment framework developed by the US Environmental Protection Agency (USEPA 1992) recommends that assessment endpoints and associated measures be used for this purpose. In this context, assessment endpoints represent components that sustain the structure, function, and diversity of an ecological system, or components that may be valued for other reasons (such as a rare species). Assessment endpoints may be identified at any level of biological organization: molecular, cellular, organism, population, community, and ecosystem (Figure 3.4). Once the assessment endpoints are selected, measures of exposure and effects can be identified. These measures reflect the actual types of data that will need to be collected in order to complete the risk assessment. Ideally, they should be able to be measured relatively easily, either indirectly or directly.

Generic assessment endpoints and measures that can be used to determine the effects of Se contamination on an ecological system were derived from the synthesis of Se research presented previously, as well as the conceptual models proposed for exposure pathways and ecological effects. Measures of exposure and effects are categorized in Table 3.1, and the measures of system characteristics are subsumed within the community- and ecosystem-level exposure and effects measures. Data collection on the key measures (in bold text) is recommended for systems where a Se problem is strongly suspected or has been identified. For systems where studies are just beginning and less information exists on whether Se is an influence, the first steps might be to measure Se concentrations in water, particulate phases (including organic carbon content of the sediment), and tissues of primary consumers.

For the purpose of characterizing Se exposure in a particular aquatic ecosystem, the recommended measures are Se concentrations in water and in biogenic particulates (used to calculate the EF) and measurement of Se concentrations in dominant primary consumers. These measures capture much of the site-specific variation in Se enrichment at the base of aquatic food webs. Temporally and spatially matched samples related to specific food webs are valuable given the site-specific nature of Se effects. The most appropriate measure of Se exposures for the purpose of estimating Se hazards to higher-order consumers is Se concentrations in eggs or mature ovaries of vertebrates (fish and/or birds), which are the best predictors of the toxic effects of Se on embryo and larval stages; measurement of Se concentrations in diets, muscle, and whole organisms are less predictive of toxic effects of Se (Chapter 7). Measurement of the biologically active species, Se-methionine, at various levels of

TABLE 3.1
**Key Assessment Endpoints and Corresponding Exposure and Effects Measures for Se Risk Assessments in Aquatic Systems. Data Collection for the Key Measures (in Boldface Type) Is Recommended for Systems Where a Se Problem Is Strongly Suspected or Has Been Identified**

| Level of Organization | Assessment Endpoint | Measures of Exposure | Measures of Effect |
|---|---|---|---|
| Molecular or cellular | Oxidative stress protection | Se in subcellular compartments | Enzyme assays and gene expression |
| | Normal biomolecule structure and function | Se substitution in biomolecules | |
| Tissue | Normal tissue structure and function | Total Se and /or selenomethionine in tissue | Pathology of liver, kidney, eyes, gills, blood, gonad |
| | | | Relative organ weight |
| Organism | Survival, growth, and reproduction of egg-laying vertebrates | **Se in female reproductive tissue of oviparous vertebrates** | Survival |
| | | | Growth |
| | | | Body condition |
| | | Se in whole-body or surrogate tissue | **Edema** |
| | | | **Embryo malformation** |
| | | | **Egg hatchability** |
| | | | Immuno-competence |
| | | | Incidence of parasites or disease |
| Population | Population sustainability | Se in diet | **Reduced abundance** |
| | | | **Population structure** |
| | | | Change in genetic diversity |
| Community | Community structure and function | **Se in water and particulates (enrichment function)** | **Presence or absence of sensitive species** and functional groups |
| | | Se speciation in particulates | Taxa richness and diversity |
| | | **Se in primary consumers** | |
| | | Trophic transfer factor | |
| | | Food web structure | |
| Ecosystem | Ecosystem structure and function | Se loading and speciation in ecosystem | Productivity |
| | | | Nutrient cycling |
| | | Residence time of Se in ecosystem | |
| | | Organic carbon in sediment | |

organization (particulates, whole-body, tissues, and subcellular components) may also provide insight into differences in Se bioavailability and toxicity among ecosystems and taxa.

The measures of effect that are most reliably diagnostic of Se toxicity in aquatic and associated terrestrial ecosystems are those most directly related to Se reproductive

toxicity at the organism level: embryo malformations (terata), embryo-larval edema, and egg hatchability. Reproductive failure can lead to effects at both the population level (reduced abundance, loss of year classes) and the community level (loss of Se-sensitive species); these changes are often the most visible evidence of Se toxicity in aquatic ecosystems. However, these measurements can be difficult to implement because of the need for a large number of samples, specialized equipment, or extensive time and resources; they also may be less diagnostic of Se toxicity because they may reflect effects of other stressors. Measures of effects at tissue and subcellular levels may be diagnostic of Se toxicity (e.g., measures of oxidative stress), but these measures are generally less predictive of effects at higher levels of organization.

## 3.8 SUMMARY

The ecological effects of Se are mediated by site-specific factors, but certain general patterns emerge from a synthesis of current research. These generalizations address the geochemistry and anthropogenic activities likely to cause risk, Se biochemistry, the cycling of Se in aquatic environments, the uptake and transfer of Se through food webs, and the mechanisms of action for Se toxicity. While recognizing that each site is different, these general patterns not only can be used to assess contaminated sites, but also to predict situations in which potential Se mobilization may cause great risk.

### 3.8.1 Selenium's Biochemical Role

Selenium is both an essential element for animal nutrition and a toxicant. In fish and birds there is a narrow margin between essentiality and toxicity. Selenium occurs in a variety of organic and inorganic forms, but selenomethionine has been associated most closely with trophic transfer and toxicity in the environment. In aquatic systems, bacteria, algae, and plants convert inorganic forms of Se into organic forms, including selenomethionine, which is then transferred through food webs and, for egg-laying species, from mother to egg. The confirmed effects of Se on reproductive success in egg-laying vertebrates, including developmental abnormalities, have been linked to vertebrate population extirpations.

### 3.8.2 Selenium as a Global Problem

Selenium is distributed globally but not uniformly in organic-rich marine sedimentary rocks. Anthropogenic activities such as coal, phosphate, and metals mining can expose Se-rich strata to greatly enhanced leaching and subsequent transport. Soils derived from weathering and erosion of Se-rich sedimentary rocks can contribute Se through agricultural irrigation runoff and drainage. Selenium also is associated with processing and combustion of fossil fuels such as coal and oil. Coal combustion and oil refinery wastes may contain greatly concentrated Se relative to the raw material, and wastes from these processes can elevate Se concentrations in aquatic environments. These and other human uses of Se-associated products can transport contamination far from sources, potentially generating problems in areas distant from source rocks. Selenium discharges and Se contamination of aquatic ecosystems can

be expected when known geologic sources of Se are combined with anthropogenic activities such as mining, irrigation, and coal-fired power plant operation unless appropriate management measures are instituted.

Specific examples of Se contamination from anthropogenic activities are well documented in the literature (Appendix A). In many of these cases, significant adverse effects on biota that are typical of Se toxicity have been documented; in some cases, population- and/or community-level effects also occurred. These case studies also demonstrate that the ecological outcome of Se contamination depends in part on measures of system characteristics such as Se loading, dissolved Se speciation, residence time or flow conditions, productivity, general food web characteristics, including diet and predator linkages, and the presence of other stressors.

Demand for coal, oil, and phosphate ore are expected to continue to increase in the foreseeable future. In addition, certain new technologies that use Se, such as nanotechnology, may have unpredicted impacts. As a result, both localized and landscape-scale Se contamination are global issues that are expected to increase in prominence in the future.

### 3.8.3 Movement and Transformation of Se

Much has been learned in recent years regarding the transport and transformation of Se in aquatic systems (Figure 3.3). Most important, research has shown that diet is the dominant pathway of Se exposure for both invertebrates and vertebrates. For this reason, traditional methods for predicting toxicity on the basis of exposure to dissolved concentrations do not work for Se. Selenium moves readily from water to primary producers and the other organic particulates that form the base of aquatic food webs. The EF, the ratio of the Se concentration in particulates to the Se concentration in water, describes the initial enrichment step for Se at the base of the food web. The EF measure in natural systems can vary by up to 2 orders of magnitude at different locations, although there is some evidence that EF values cluster more closely among sites with similar characteristics (e.g., lake systems versus river systems). This variability in EF makes it difficult to predict Se exposure and effects from water concentrations alone.

Transfer from particulates to primary consumers is less variable. TTFs (ratio of Se concentration in consumers to Se concentration in diet) for invertebrates are site and species-specific, but generally vary within 0.6 to 23. This dietary pathway is dominant; uptake of Se directly from water by consumers is negligible. Similarly, transfer from invertebrates to fish is from 0.6 to 1.7. For these reasons, the composition of the food web is important in determining bioaccumulation; the length of the food chain does not necessarily predict the level of Se exposure.

### 3.8.4 Effects of Se on Ecosystems

Acute toxicity from exposure to elevated dissolved Se concentrations has rarely, if ever, been reported in the aquatic environment. Significant chronic effects would be expected at far lower dissolved Se concentrations due to the incorporation of Se into the food web and resulting exposure and toxicity to fish and birds.

Chronic Se toxicity is manifested through reproductive impairment via maternal transfer in egg-laying vertebrates, resulting in embryotoxicity and teratogenicity. Other chronic effects include reductions in growth, tissue pathologies, induction of oxidative stress, and mortality. Sensitivity to chronic Se toxicity may vary widely, even among closely related species. Because estimates of risk are developed from knowledge of exposure and effects, the species that are most sensitive to Se are not always the most exposed to Se in nature. Species-specific feeding habits that result in high exposure levels may also drive toxicity risks. While much has been learned about bird and fish species, far less is known about toxicity in other oviparous vertebrates. A notable knowledge gap exists for egg-laying species of amphibians and reptiles, which include some of the most critically endangered vertebrate species.

Direct effects of Se on the population and community levels of biological organization have been documented at some sites (Appendix A). There is much less information about ecologically relevant indirect effects at the community or the ecosystem levels. Changes in invertebrate community structure caused by Se-induced loss of fish predators could be one example. Interactions between Se and temperature or other stressors also may occur but require further study.

These observations help explain why the behavior and toxicity of Se in ecological systems are highly dependent on site-specific factors. Knowledge of the food web is one of the keys to determining which biological species or other ecological characteristics will be affected. Other important parameters include rates of input of Se into the system, hydraulic residence time, and Se speciation in water and particulates.

It is difficult to generalize about system recovery when Se contamination is reduced or removed. Recovery is a function of the characteristics of the particular ecosystem and the decreases in mass loading of Se. Experience at Belews and Hyco Lakes shows that, once the source is removed, aquatic communities can substantially recover within a few years, although the community composition may be altered. Selenium in sediment may contribute to long (decadal) recovery times of tissue residues and possible long-term persistence of adverse effects in aquatic consumers.

### 3.8.5 How to Investigate a Potential Se Problem

Key assessment endpoints and corresponding exposure and effects measures at multiple levels of biological organization can be used to diagnose a suspected Se problem (Table 3.1). Similar assessment endpoints and measures also can be used to help predict potential impacts of a future anthropogenic activity.

Based on current knowledge, the endpoints most diagnostic of Se exposure occur at the tissue and organism levels. Table 3.1 presents the key measures recommended for assessing an ecosystem where significant Se contamination is strongly suspected or known. In systems where Se contamination is less certain, a shorter list of initial endpoints is proposed that includes Se concentrations in water, particulates, reproductive tissues from oviparous fish and wildlife, and tissues from primary consumers. In either situation, significant insight into the fate and effects of Se also may

be gained by evaluating system characteristics such as Se loading and speciation, hydraulic residence time, ecological productivity, general food web characteristics, and the presence of other anthropogenic or natural stressors.

## 3.9 PRIORITIES FOR FUTURE RESEARCH

Selenium research has progressed in recent decades and has resulted in significant advances in our knowledge of Se dynamics and effects in aquatic systems. There are still important unknowns, however, and we suggest the following priorities for continued research:

1. Determine the species sensitivity of other egg-laying vertebrates, including reptiles and amphibians.

   Research has confirmed the susceptibility of oviparous fish and birds due to the maternal transfer of Se, and subsequent embryonic effects. There is insufficient toxicity information (in some cases, no toxicity information) on other oviparous species, including reptiles and amphibians.
2. Synthesize information regarding methods for collection of particulate components and develop a database of EF values.

   Particulate Se determines the uptake of Se into the base of the food web and serves as the Se source for primary consumers. There is substantial variability in approaches to particulate matter definition, collection, and analysis.
3. Obtain more information on Se sensitivity of marine species.

   There is insufficient information on Se effects in marine organisms.
4. Expand biodynamic modeling in freshwater systems.

   Collection of additional data regarding relationships among environmental compartments should lead to more reliable predictions of exposure and effects in freshwater systems. This would include more generalizable relationships across systems.
5. Develop additional quantitative surrogates for reproductive endpoints.

   Because it may be difficult or impractical to measure reproductive endpoints directly, alternative approaches would be valuable. For example, if a confirmed, quantitative relationship between diet and a reproductive endpoint is established, data on diet can then be used to predict reproductive toxicity risk.
6. Elucidate the mechanisms of Se toxicity.

   Although selenomethionine appears to be the form of Se that is most closely associated with adverse reproductive outcomes in wildlife, the precise mode of action for these toxic effects is poorly understood.
7. Explore indirect effects of selenium exposure within ecological systems.

   An understanding of changes in ecosystem ecological structure due to Se exposure is needed, including system-wide effects mediated via loss of food resources, disruption of predator–prey relationships, and loss of predators.

8. Identify interactive effects of selenium with other contaminants and stressors.

   Future studies on Se toxicity should consider the possible interactions between Se and common ecological variables (e.g., temperature, salinity, climate), important events in an animal's life history (e.g., migration, metamorphosis), and other anthropogenic factors (e.g., eutrophication, habitat modification, interactions with other contaminants). Although it is well known that Se interacts with other elements such as Hg, much remains to be known about the molecular mechanisms driving these interactions and their implications for toxicity.

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