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ECOTOXICOLOGY
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CHAPTER 17

Ecotoxicology of Selenium

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CONTENTS

17.1 INTRODUCTION

Selenium is a naturally occurring semimetallic (also referred to as a metalloid) trace element that is essential for animal nutrition in small quantities but becomes toxic at dietary concentrations that are not much higher than the required levels for good health. Thus, dietary selenium concentrations that are either below or above the optimal range are of concern. This chapter summarizes the ecotoxicology of excessive selenium exposure for animals, especially as reported during the last 15 years. It focuses primarily on freshwater fish and aquatic birds because fish and birds are the groups of animals for which most toxic effects have been reported in the wild. However, information related to bioaccumulation by plants and animals as well as effects in invertebrates, amphibians, reptiles, and mammals also are presented.

Selenium was recognized long ago as a cause of toxicity in domestic poultry and livestock (e.g., References 1–3), although a recent reevaluation of some of the historic samples and related information by O'Toole and Raisbeck[4] indicates that the condition known as "blind staggers" in livestock is probably not caused by selenium. For fish and wildlife, selenium became much more of a concern in the 1970s and 1980s with the discovery of selenium bioaccumulation and severe impacts in fish and aquatic birds. These problems and other general information (such as environmental occurrence, general and biochemical properties, and other characteristics) are summarized in a number of recent reviews and books about selenium (see References 5–19) and are reviewed briefly in this chapter. Readers may wish to consult those references for additional information.

As a result of new information related to food-chain bioaccumulation and effects of selenium in freshwater fish, the ambient water quality criteria were revised in 1987,[20] and they are being reviewed again.[21,22] Water quality criteria for freshwater systems are based on total recoverable selenium (as discussed later in this chapter), whereas the saltwater criteria are based on dissolved selenium. Because of differences in the toxicity of different chemical forms, the current acute criterion (criteria maximum concentration) for freshwater considers selenite and selenate as relative fractions of the total recoverable selenium concentration.

The environmental significance of selenium is a topic of ongoing discussion and debate. A recent issue of the journal *Aquatic Toxicology* (Vol. 57, No. 1) was devoted to papers on selenium. In December 1999, the journal *Human and Ecological Risk Assessment* devoted an issue (Vol. 5, No. 6) to debate and commentary on the topic, "Selenium — A potential time bomb or just another contaminant?" and published several papers on the topic. Needless to say, the debate continues, as not all the issues were resolved. In particular, there are differences of opinion concerning waterborne concentrations of selenium that are protective for fish and wildlife,[23–28] the relative importance of sediment vs. waterborne selenium in affecting aquatic biota,[29–31] and the specific thresholds of selenium in the diet or eggs of birds at which reproduction is adversely affected.[32–34] These topics are discussed in later sections of this chapter.

Selenium concentrations typically are reported as micrograms per liter (μg/L) in fluids and milligrams per kilogram (mg/kg) or micrograms per gram (μg/g) in soil, sediment, plant or animal tissues, and diets. Concentrations in soil, sediment, tissues, and diets can be expressed either on a

wet-weight or fresh-weight basis (which are considered to be synonymous), or on dry-weight basis. Conversion from one basis to the other is a function of the moisture content in the sample (which should be reported regardless of which basis is used), as follows:

$$\text{Dry-weight conc.} = \text{Wet-weight conc.} \times \frac{100}{(100 - \text{Moisture percentage})}$$

For example, 10 mg/kg on a wet-weight basis in a sample having 80% moisture is equal to 50 mg/kg on a dry-weight basis. Selenium concentrations in soil, sediment, and tissue throughout this chapter are given on a dry-weight basis, unless otherwise noted.

17.2 ENVIRONMENTAL CHEMISTRY

From an ecotoxicological perspective, it is important to have an understanding of selenium's chemical characteristics, environmental sources and occurrence, and cycling in the environment. Those aspects of selenium chemistry can help define geographic areas and environmental settings in which selenium may be a chemical of ecological concern, and they are briefly summarized in this section.

17.2.1 Chemical Characteristics

Selenium chemistry is complex, and chemical forms vary in their environmental occurrence, biogeochemistry, and toxicity.[7,15,17,19,35–38] In nature, selenium is generally recognized to occur in four oxidation states: selenide (Se^{2-}), elemental selenium (Se^{0}), selenite (Se^{4+}), and selenate (Se^{6+}), although a fifth form (selenium dioxide [Se^{2+}]) may occur as a product of combustion of elemental selenium present in fossil fuels or rubbish.[19] Soluble selenates are the predominant form under oxidizing conditions in alkaline soils that are commonly found in arid areas. Selenates are readily available to plants, or they can be slowly reduced to selenites, which also can be taken up by plants and converted into organic forms. The common organic forms in plants include selenomethionine, selenocysteine, dimethylselenide, and dimethyldiselenide. Selenite is the more common soluble form of selenium under reducing conditions and in acidic soils, which occur more typically in higher-rainfall areas. Selenium occurs in various organic and inorganic forms in coal and other fossil fuels. In general, elemental selenium and metallic selenides are not readily bioavailable. However, under oxidizing conditions they can be transformed to bioavailable forms.

Selenium commonly occurs as a mixture of several different chemical forms in surface waters, but selenates and selenites are most common.[10,17,25,38] Metal and organic selenides are common in bottom sediments. Waterborne selenium can be evaluated most reliably as total recoverable concentrations,[17] and total recoverable concentrations are the basis of the ambient water quality criteria for freshwater ecosystems.[20,22] This is because partitioning of selenium between the water column and other compartments of an aquatic ecosystem greatly affects the measurement of waterborne selenium. Low waterborne selenium concentrations can reflect low mass loading of the element, but they also may reflect high biotic uptake[17] (see also Kesterson Reservoir example below). Total recoverable selenium includes suspended detrital particulate matter (a function of biotic uptake) and thus more accurately reflects the total mass load of selenium in the system. Differences between dissolved and total recoverable measurements increase with higher levels of eutrophication of the water body.

During the early and mid-1980s, subsurface agricultural drainage waters from the San Joaquin Valley, California, were disposed of by discharging them to Kesterson Reservoir, which was a series of 12 shallow ponds totaling about 500 ha.[7] Selenium concentrations in water entering the reservoir during 1983 to 1985 averaged about 300 μg Se/L, and aquatic plants and invertebrates contained

greatly elevated concentrations of selenium (typically 10 to 100 times those found at a nearby reference site).[7,39–42] Almost all of the waterborne selenium was in the selenate form. Unlike boron and a number of other constituents, selenium concentrations *decreased* as the water flowed through the series of ponds and evaporated. Similarly, plants and animals as well as sediments in ponds nearer the inflow contained higher concentrations of selenium than those downstream. Thus, bioaccumulation by plants and animals removed substantial amounts of selenium from the water and deposited it into the sediments. Similar findings were reported for another site in Montana.[43]

Selenium is essential for animals and for some plants.[19] Biochemically, it is similar to sulfur; when selenium is present at elevated dietary levels, it replaces sulfur in some metabolic pathways and thereby causes problems[23,36,37,44] (see also, later section on effects in fish). Selenium is an essential component of the enzyme glutathione peroxidase, which, along with vitamin E, serves as an antioxidant to prevent metabolic damage to tissues.

Selenium occurs in various chemical forms in plant and animal tissues, but bioavailability is greater from plant selenium than from animal foods.[5,45] Selenomethionine has been found to be a good surrogate for natural food-chain selenium in experimental studies.[17] In general, the diet is the most important exposure pathway for vertebrate animals and should be included when conducting or evaluating the effects observed in experimental studies.

17.2.2 Environmental Sources and Occurrence

Typical concentrations in the Earth's crust are < 0.5 mg Se/kg, but some geologic formations are greatly enriched in selenium.[19,36,39,46–50] Especially when these formations are modified as a result of human activities (e.g., mining, agricultural irrigation), the selenium may be mobilized and become more available to plants and animals, which bioaccumulate selenium and incorporate it into the food chain for other organisms. Selenium tends to be present in large amounts in areas where soils were derived from Cretaceous and Tertiary marine sedimentary rocks, and selenium from these sources is highly mobile and biologically available in arid regions having alkaline soils.[17]

Industrial sources, such as coal and oil combustion, nonferrous metal production (primarily copper and nickel, but also lead, zinc, and cadmium), steel and iron manufacturing, municipal and sewage refuse incineration, and production of phosphate fertilizers, introduce much more selenium into the environment than do natural sources such as volcanic activity or weathering of seleniferous rocks.[51,52] However, there are no ores from which selenium can be mined as a primary product.[53] It is found mainly in sulfide minerals of copper, iron, and lead and is most commonly produced by electrolytic separation from copper during the refining process. Average annual usage is estimated at 1850 metric tons. Selenium is used in a wide variety of industrial applications, with glass (35% of demand) and electrical (30%) applications representing most of the use. Other uses include pigments (10%), metallurgy (10%), agricultural/biological (5%), and miscellaneous applications (10%).

Fossil fuels (coal and oil), as well as associated formations from which they are extracted, may contain elevated concentrations of selenium.[19,25] Concentrations vary considerably depending on the source. The selenium may be mobilized through mining of coal or disposal of fly ash from power plants where the coal was burned. In terrestrial environments, when fly ash is deposited in landfills, it may be taken up in significant amounts by plants growing on the landfill.[18] One well-known example of effects associated with fly ash disposal into water bodies has been reported in a series of studies conducted at Belews Lake, North Carolina (see, for example, Reference 24). Similarly, selenium from crude oil may be discharged with process wastewater from the refinery, thereby contributing to bioaccumulation by organisms in the receiving waters.[25,54]

Mass-loading of selenium into aquatic environments most typically results from disposal of coal fly ash, irrigation wastewater, or oil refinery wastewater.[17,25,54] Mining or smelting of sulfide ores, uranium, phosphate, bentonite, and coal also contributes to mobilization of selenium because of its presence in the mined materials or the overburden that is moved during the mining process.[17,19,47,55]

In freshwater systems, normal background levels of selenium in sediment are 0.2 to 2.0 mg/kg, and in water 0.1 to 0.4 μg/L.[17]

17.2.3 Cycling

Selenium reaches the earth's surface through volcanic activity, and some authors have proposed that oceans were enriched in selenium from volcanic activity during Cretaceous times.[7,35,46,50,56] This early enrichment, along with bioaccumulation of selenium in the oceans and later deposition in the sediments,[48] would explain the high selenium concentrations found in many Cretaceous marine rocks. Tertiary marine sedimentary rocks contain selenium resulting from bioaccumulation and deposition of particulate matter derived by erosion of older (Cretaceous) seleniferous sedimentary deposits and also are generally seleniferous.

Irrigation of seleniferous soils can dissolve and mobilize selenium and then transport it in groundwater to irrigation drains.[48–50] Geology, climate, and hydrology were found to be important factors affecting the mobilization and transport of selenium into habitats where biota are adversely affected in the western United States. Although selenium can be transported naturally as a result of rock weathering and drainage into water bodies, the process has been increased through irrigation of soils derived from seleniferous formations, especially Cretaceous and Tertiary marine sedimentary deposits.

Many biogeochemical processes affect the cycling of selenium through different components of the environment.[7,10,35,52,57,58] In wetlands, selenium oxidation-reduction reactions are the most important processes controlling speciation, precipitation/dissolution, sorption/desorption, methylation, and volatilization of selenium. Selenate is stable in well-oxidized environments, but it is converted slowly to selenite or elemental selenium under less-oxidized conditions. It can be further reduced to metal selenides or volatile methylated forms (primarily dimethylselenide) through microbial processes. The metal selenides tend to be deposited in the wetland sediments, whereas volatile forms escape to the atmosphere. Selenides and elemental selenium tend to be very insoluble, although they can be converted to more soluble forms (selenite and selenate) under oxidizing conditions.

The changes described above can be important considerations in the wetting and drying cycles that occur in seasonal wetlands, as well as periodic draw-down of water levels in permanent wetlands. When submerged, especially where large amounts of organic material are present, selenium tends to be present in reduced (and less toxic) forms, and volatilization is favored.[10] If the water level is lowered, the selenium becomes more oxidized and bioavailable. Thus, the selenium present in the wetland sediments and organic matter would become more likely to bioaccumulate into aquatic organisms soon after the wetland is reflooded than when it was previously flooded.

Although selenium in igneous and sedimentary rocks may be insoluble and unavailable to terrestrial plants, chemical weathering and plant and microbial action transform much of it into soluble (and bioavailable) forms.[46,56] Oxidation of this selenium in alkaline soils produces selenate, making it readily available to plants. Selenium uptake by plants is affected by both the concentration and its bioavailability in soil. Selenium uptake by plants is greater in soils having higher pH and in areas of low rainfall. In areas with acidic soils and higher rainfall, selenium is generally less available to plants for uptake.

Selenium enters the atmosphere through volcanic activity, burning of fossil fuels (especially coal), and volatilization from terrestrial or aquatic environments.[35,52] Although concentrations are low (typically around 1 ng/m^3 air, but ranging up to 6000 times that level near a smelter[59]), atmospheric dispersion and subsequent deposition of the selenium redistributes it in the environment. For example, deposition is estimated to have contributed to a 15% increase in soil selenium in the United Kingdom during the last century and to contribute between 33 and 82% of plant leaf selenium uptake. Deposition occurs either as "wet deposition" (with precipitation), which accounts for most of the deposition, or "dry deposition" (exchange of particulate and gaseous material between the atmosphere and the global surface).

Numerous studies of selenium transformations, cycling, and volatilization in aquatic and terrestrial ecosystems have been reported (or reviewed) in recent years.[16,18,43,52,60–67] Not surprisingly, rates for these processes vary greatly, depending on temperature, moisture, organic carbon content of soil/sediment, selenium concentration and chemical form, and microbiological activity. Although details of some of the processes are not yet well known, it is clear that microbes are largely responsible for many of the changes that occur.

Plants vary widely in their ability to accumulate and volatilize selenium from contaminated wastewater, soils, and sediments.[7,66,68] Under some circumstances, phytoremediation of these contaminated media may be a viable approach to reducing environmental exposures. However, waterborne selenium can be accumulated by plants and animals living in the wetlands to levels that are harmful to aquatic organisms as well as birds that feed upon them.[7,19] As seleniferous waters enter a wetland, selenium is removed from the water through a combination of bioaccumulation by plants and animals, deposition in the sediments, and volatilization (as relatively low-toxicity dimethylselenide) to the atmosphere.[40,42,69,70] Because of selenium bioaccumulation in the food chain and deposition to sediments, wetlands should not be used for phytoremediation of selenium-contaminated wastewater without conducting an ecological risk assessment to evaluate potential adverse effects to birds and other potentially exposed animals.

The biological half-life of selenium in animals is relatively short, varying from 10 days in pheasants to 64 days in earthworms.[71,72] More detail concerning uptake and loss of selenium in birds is presented in the section on bioaccumulation in birds.

17.3 BIOACCUMULATION

Selenium bioaccumulates in aquatic and terrestrial food chains and in higher trophic-level animals that feed on those plants and animals. Bioaccumulation is defined as the combined net accumulation (taking into account any concurrent loss) of a chemical from abiotic media and ingestion of selenium-containing biota (i.e., the food chain). In aquatic systems, bioaccumulation may occur by direct absorption or partitioning from water or sediment or by ingestion. The greatest level of bioaccumulation is typically from water to aquatic plants or invertebrates (often a 1000-fold or greater increase occurs). In terrestrial systems, bioaccumulation is primarily from ingestion, because uptake from air is less than that from water, and airborne concentrations generally are lower than those in water. When concentrations in animals are considered on whole-body basis (which is the appropriate perspective), selenium does not biomagnify through the various trophic levels. (Biomagnification is defined as increasing concentrations through successive trophic levels.) Nevertheless, significant bioaccumulation does occur (as described in this section) and leads to adverse effects in sensitive organisms (especially fish and birds; see next section).

This section presents a general summary of selenium bioaccumulation by plants and animals. Selenium concentrations typically are higher in marine organisms than in those from freshwater ecosystems, and they are higher in areas having seleniferous soils or sediments and in areas receiving industrial, agricultural, or municipal wastes. Additional information, including more detailed tabulations of selenium concentrations in biota, are provided by Jenkins,[73] Wilber,[71] and Eisler.[19]

17.3.1 Plants

17.3.1.1 Aquatic

Background concentrations in freshwater algae are 0.1 to 1.5 mg Se/kg, and in rooted plants they are 0.1 to 2.0 mg Se/kg.[17,19] By comparison, algae and rooted aquatic plants accumulated 20 to 390 mg Se/kg in Kesterson Reservoir, California, which received agricultural drainage waters containing up to 330 μg Se/L.[40–42] Selenium bioaccumulated in aquatic plants 28 to 5100 (mean =

1105) times the concentration in water.[42] Elevated concentrations of selenium also have been found in plants from ponds receiving coal fly ash.[74, 75]

The bioaccumulation and volatilization of selenium by wetland plants (referred to as phytoremediation) can be used to treat waterborne selenium under certain conditions. There was at least a 50-fold variation in selenium accumulation and volatilization among 20 aquatic species tested with water containing selenite or selenate.[66] Several of the aquatic plant species showed selenium volatilization and accumulation rates that were similar to that of Indian mustard (*Brassica juncea*), the best-known terrestrial plant species for phytoremediation. However, the overall implications of using vegetated wetlands to remove selenium from agricultural or industrial wastewaters are not yet well studied. This technology may be appropriate for waters containing moderate levels of selenium (perhaps up to 25 or 30 µg/L), but only if it is designed and managed to limit access by aquatic birds that would feed on plants and invertebrates in the wetland. However, at higher waterborne concentrations of selenium, or with inadequate attention to design and management concerns, the wetland could present undesirable levels of exposure to birds.

Several laboratory microcosm studies (summarized below) have measured selenium bioconcentration (i.e., direct uptake) by algae from water and subsequent transfer of selenium to invertebrates or fish consuming the algae. These studies show that the chemical form of the waterborne selenium is an important factor in affecting uptake by aquatic biota and that sulfate content of the water can influence uptake. These and other similar laboratory studies of aquatic systems show that selenium bioaccumulation is a significant mechanism of concern for higher trophic-level organisms.

Bioconcentration factors (BCFs) from water to macrophytes (rooted plants and filamentous algae), periphyton (algae and associated material attached to the wall of the microcosm), and zooplankton (daphnids and their offspring) were significantly higher for selenomethionine than for selenite, which in turn had higher bioconcentration factors than selenate.[76] Volatilization of selenium from the microcosms followed the same pattern (i.e., highest for selenomethionine at 24% loss during the 28-day exposure). For all three forms of selenium, bioaccumulation was higher in zooplankton than in periphyton, which had greater accumulation than macrophytes.

Another study by Besser et al.[77] with algae (*Chlamydomonas reinhardtii*), daphnids (*Daphnia magna*), and fish (bluegill, *Lepomis macrochirus*) also showed higher bioaccumulation from waterborne selenomethionine than from either selenite or selenate. Algae and daphnids concentrated selenium more strongly from selenite (BCFs = 220 to 3600) than selenate (BCFs = 65 to 500), whereas bluegills concentrated selenium about equally from both inorganic forms (estimated BCFs = 13 to 106). Bioaccumulation of foodborne selenium by daphnids and bluegills was similar in food chains dosed with different forms of selenium, and they did not accumulate selenium concentrations greater than those in their diet (except at very low dietary selenium concentrations). In exposures based on selenite, bluegills accumulated greater selenium concentrations from food than from water, and aqueous and food-chain uptakes were approximately additive. The results indicate that most of the accumulation of inorganic waterborne selenium by bluegills in selenium-contaminated environments is via food uptake. However, organoselenium compounds, such as selenomethionine, may contribute significantly to selenium bioaccumulation by bluegills through both aqueous and food-chain uptake.

Corixids (*Trichocorixa reticulata*), which are common insects in wetlands receiving agricultural drainage waters and are important food-chain organisms for fish and birds, were exposed to waterborne and foodborne selenium.[78] Algae (*Oscillatoria* sp. filaments and a *Microcystis*-type colonial alga), the food for the corixids, were exposed to water containing sodium selenate at selenium concentrations ranging from 7.3 to 870 µg/L for 48-h exposures. Corixids exposed to waterborne selenate did not accumulate selenium above control concentrations. Corixids fed algae exposed to ≥87 µg Se/L selenate had significantly higher selenium concentrations than controls, suggesting that corixids may be isolated from the water and that selenium accumulation is solely through dietary exposure.

Transfer of selenium also was followed through a laboratory food chain (water-algae-rotifers-larval fish), and its effects on the larval fathead minnows (*Pimephales promelas*) were evaluated.[72]

Selenium uptake was measured in rotifers (*Brachionus calyciflorus*) fed algae (*Chlorella pyrenoidosa*) that had been cultured in a selenate-containing medium. The rotifers were then fed to larval fathead minnows, and uptake and loss of selenium as well as effects in the fish were measured. Selenium concentrations in the fish (51.7 and 61.1 μg/g in two experiments) were similar to those in their food (68 and 55 μg/g). Final weights of larvae fed selenium-contaminated rotifers were significantly lower than those of controls, although mortality did not occur. Biological half-life of food-derived selenium in the fish larvae was 28 days.

Selenium concentrations in field-collected widgeongrass (*Ruppia maritima*) varied among samples from four evaporation pond systems in the San Joaquin Valley, California.[79] These plants were used as a substrate to measure selenium bioaccumulation and effects in a laboratory benthic-detrital food chain with midge (*Chironomus decorus*) larvae. Following a 96-h exposure, selenium concentrations had increased significantly in the three groups of larvae exposed to plants having the highest selenium concentrations, but there was no consistent pattern of adverse effects on larvae weights. After a 14-day exposure, selenium bioaccumulation patterns in midge larvae were similar to that observed after 96 h. However, as midge selenium concentrations increased, mean weight decreased, indicating that selenium apparently had a significant effect on growth. The results of this study complement other findings that indicate sediment and the detrital pathway are important in the cycling of selenium in aquatic systems.[80–82]

Waterborne sulfate reduces the uptake of selenium by plants when waterborne selenium is in the selenate form. Increasing sulfate resulted in significantly reduced selenate uptake by algae (*Selenastrum capricornutum*) and increased algal growth.[83] Sulfate also reduced selenium uptake from waterborne selenate by widgeongrass, but selenite and selenomethionine uptake were not affected by sulfate.[84] As in other studies summarized above, uptake of selenomethionine (BCFs up to 21,800) by widgeongrass was much higher than for selenite or selenate, and the highest rates of uptake (i.e., BCFs) occur at the lowest waterborne concentrations. Results of this and other field and laboratory studies show that waterborne selenium occurs under field conditions in a mixture of chemical forms, and BCFs therefore vary depending on the environmental conditions and sources of selenium.

17.3.1.2 Terrestrial

Background selenium concentrations in terrestrial plants are 0.01 to 0.6 mg/kg.[17,85] Bioaccumulation of selenium by plants depends on the species of plant, environmental conditions, age and phase of plant growth, and the chemical form of selenium present.[3,36,46,85,86] Some plants, such as white clover (*Trifolium repens*), buffalograss (*Buchloe dactyloides*), and grama (*Bouteloua* spp.), growing on seleniferous soils accumulate surprisingly low levels of selenium. In contrast, high sulfur-containing plants like the *Brassica* species (mustard, cabbage, broccoli, and cauliflower) and other Brassicaceae are relatively good accumulators of selenium.

Three groups of plants are generally recognized on the basis of their tendency to accumulate selenium when grown on high-selenium soils (as originally described by Rosenfeld and Beath[3]). The first two groups of plants are referred to as *selenium accumulator* or *indicator* plants. These plants grow well on soils containing high levels of available selenium, and some have been used to locate seleniferous soils. Plants in Group 1 are called primary indicators or accumulators and normally accumulate selenium to very high concentrations (often several thousand mg/kg). Plants in Group 2 are referred to as secondary accumulators and may contain a few hundred mg Se/kg in their tissues. Those in Group 3 include grains, grasses, and many other plants that do not normally accumulate selenium in excess of 50 mg/kg when grown on seleniferous soils. Deep-rooted shrubs and other plants with long tap roots may act as pumps, bringing selenium from the deeper soil profiles to the surface and near-surface soils, where it is available to shallower-rooted plants such as grasses.[87]

Selenium concentrations in plants usually decline with maturity, so the highest concentrations usually occur during the most active growth phase.[3,85] Because selenium is associated with protein in the plant, leaves usually contain higher selenium concentrations than seeds (e.g., see also Schuler

et al.[42]). Although plant selenium concentration can generally be used as an indicator of soil selenium status,[85] total selenium concentrations in soil do not necessarily determine whether the plants growing there will induce toxicity or nutritional deficiency in animals.[88]

Following the discovery of significant effects of selenium in aquatic birds feeding at Kesterson Reservoir, California, inflow of agricultural drainage water was halted, the reservoir was dewatered, and lower-elevation portions of the site were filled with soil to prevent groundwater from rising to the ground surface.[89,90] Loss of this wetland habitat was mitigated through wetland enhancement in nearby areas. These actions converted the site to a mosaic of three terrestrial habitats, including areas that were filled, those higher-elevation areas where existing vegetation (mainly cattail, *Typha* sp.) was disked, and other higher-elevation grassland areas where no action was taken. Further descriptions of the site and its early history are provided by Ohlendorf[7] and Ohlendorf and Hothem.[91]

Biological monitoring of Kesterson Reservoir has been conducted annually since 1988 and has included extensive sampling and analyses of soil (for total and water-extractable selenium), plants, invertebrates, birds (mainly their blood or eggs), and mammals.[89,90] The chemical behavior of selenium in the Kesterson environment has been characterized through studies of selenium speciation and fractionation in soils, long-term monitoring of the spatial and depth distribution of selenium in soil, field-measured volatilization rates from several experimental plots, laboratory measurements of selenium reoxidation rates under controlled conditions, modeling studies of the above-mentioned data to determine reoxidation and leaching rates, and bioaccumulation by plants and animals at the site. This information was integrated to conduct ecological risk assessments in 1993 with the limited data set available at that time and again with a much larger data set in 2000.[89,90]

The key factor in the exposure model for the site is the water-extractable selenium concentration in soil, which influences bioaccumulation by plants that form the base of the food chain. Although there is a highly significant ($P < 0.001$) increase in plant selenium with increasing water-extractable soil selenium, the relation between the two matrices is highly variable.[89,90] Selenium concentrations vary somewhat from year to year and among plant species. For example, in 1995, the selenium concentration ranged from 0.20 to 90 mg/kg in 286 samples, with a geometric mean of 3.4 mg/kg; in 1998, the range in 240 samples was 0.20 to 68 mg/kg, with a geometric mean of 3.8 mg/kg.

Uptake and accumulation of selenium by plants growing on fly-ash landfills has been studied extensively and reviewed recently.[18] Concentrations of selenium in vegetation vary by plant species, with legumes typically accumulating more selenium than grasses. Although some other elements (such as boron and molybdenum) also bioaccumulate in these plants, this does not occur to elevated concentrations as consistently as the bioaccumulation of selenium. Application of sulfur in the form of gypsum reduced the bioaccumulation of selenium in several plant species. Plants growing on fly-ash landfills can transport selenium to the surface and make it available to the surrounding environment. Reported concentrations of selenium in plants growing on or near these landfills are typically less than 10 mg/kg, although concentrations exceeding that level have been found in grass (*Phalaris arundinacea*), trees (*Populus deltoides, Salix* spp.), and herbaceous vegetation growing at the margin of an ash-settling pond and in plants growing in fly ash without soil cover.

17.3.2 Invertebrates

17.3.2.1 Aquatic/Marine

Background concentrations in aquatic invertebrates are 0.4 to 4.5 mg Se/kg, although concentrations are typically less than 2 mg Se/kg.[12,17,19] Elevated concentrations have been reported in freshwater and estuarine invertebrates from areas receiving subsurface agricultural drainage,[40–42,82,92] urban/industrial discharges,[93] and in ponds or lakes that received selenium-containing fly ash.[75,94–96]

Among the aquatic invertebrates sampled at Kesterson Reservoir, California, selenium concentrations were highest in benthic species, such as midge larvae (Chironomidae) and dragonfly and damselfly nymphs (Odonata), and lowest in water boatmen (Corixidae).[40–42] Mean concentrations

in some of the invertebrates from Kesterson Reservoir exceeded 100 mg Se/kg. Selenium concentrations in adult damselflies were significantly higher than in nymphs.[41] Selenium bioaccumulation factors for invertebrates ranged from 168 to 3700 (mean = 1090).[42] Most aquatic insects contained lower concentrations than rooted aquatic plants but higher concentrations than water, sediment, algae, and diatoms.

Midge larvae show small-scale spatial variability in selenium accumulation,[97] which probably reflects variability in concentration and bioavailability of selenium in sediment and perhaps the ages of the larvae. Grain size and organic carbon content can influence sediment selenium concentration and bioavailability. Consequently, when sampling/monitoring is being conducted to characterize dietary exposure of fish and aquatic birds that feed on midge larvae, it is important to analyze randomly collected, composited samples taken throughout the potential feeding area.

Laboratory experiments have indicated that 96% of selenium in mussels (*Mytilus edulis*) was obtained from ingested food, with much less accumulation directly from water than for several other elements.[98] Several other laboratory studies of the relative bioaccumulation in freshwater aquatic organisms from waterborne selenite, selenate, and selenomethionine are discussed above in the section on plants. At selenate concentrations greater than 5 µg Se/L, selenium uptake by daphnids (*Daphnia magna*) was inversely related to waterborne sulfate concentration.[99] Similar effects were reported for aquatic plants, as described above.[83,84]

17.3.2.2 Terrestrial

Background concentrations in terrestrial invertebrates are 0.1 to 2.5 mg/kg.[17] Earthworms were found to bioaccumulate elevated concentrations of selenium from selenite-enriched soil (up to 7.5 mg Se/kg, fresh weight[100]), and earthworms from soil amended with sewage and those from a control field contained 15 to 22 mg Se/kg (dry weight).[101]

Extensive sampling of terrestrial invertebrates (various kinds of insects as well as spiders) has been conducted at Kesterson Reservoir, California since its conversion to terrestrial habitats in 1988 (described above and in Ohlendorf and Santolo[89]). From 1989 to 1995, samples were collected annually, and sampling was conducted again in 1998.[90] Generally, selenium concentrations in carnivorous invertebrates (such as ambush bugs, scarab beetles, and spiders) were significantly higher than those in noncarnivorous insects (such as herbivorous beetles, crickets, and grasshoppers). For example, the geometric mean for 35 samples of carnivorous invertebrates in 1995 was 18 mg Se/kg (range 4.5 to 48 mg Se/kg), and the geometric mean for 110 samples of noncarnivorous insects was 7.4 mg Se/kg (range 0.60 to 40 mg Se/kg). In 1998, the geometric mean for 35 samples of carnivorous invertebrates was 17.1 mg Se/kg (range 8.1 to 36 mg Se/kg), and the geometric mean for 104 samples of noncarnivorous insects was 12.8 mg Se/kg (range 1.8 to 61 mg Se/kg).

17.3.3 Fish

17.3.3.1 Freshwater

Background concentrations in livers of freshwater fish are 2 to 8 mg Se/kg, and in other tissues they are 1.0 to 4.0 mg Se/kg.[17] In the National Contaminant Biomonitoring Program, sampling of freshwater fish was conducted five times throughout the United States during the period 1976 to 1986.[102] Geometric means for whole-body fish were between 0.4 and 0.5 mg Se/kg wet weight (or about 1.6 to 2.0 mg/kg dry weight) in all years, with the highest mean in samples from 1976 to 77. The 85th percentile values were 0.66 to 0.82 mg Se/kg wet weight (about 2.6 to 3.3 mg/kg dry weight). These values were typically exceeded at several stations, most of which are in arid areas of the western United States in basins containing substantial amounts of irrigated agriculture. One exception to that pattern is the Waikele Stream site at Waipahu, Hawaii, where the 85th percentile values were regularly exceeded.

Elevated levels of selenium have been reported in fish primarily from seleniferous areas of the western United States and from reservoirs contaminated by fly ash from combustion of coal.[14,19,25,40,41,96,103–108] Typical patterns found in these fish (and those sampled elsewhere) are that selenium concentrations in muscle tissue are considerably lower than in livers, gonads, or whole-body fish. Selenium concentrations in gonads (and especially the eggs) as well as whole-body fish provide the best basis for evaluating exposure of the fish to selenium and the potential for adverse effects in the fish or their reproduction (as discussed in a later section). However, it is possible, especially if the fish of concern is an endangered species, to evaluate exposure through nonlethal sampling of muscle plugs or eggs so the fish is not sacrificed for analysis.

Mosquitofish (*Gambusia affinis*) from the selenium-contaminated Kesterson Reservoir contained exceptionally high concentrations of selenium.[7,40,41] Fish were analyzed as composite samples of many individuals taken from different portions of the site, and concentrations were compared to those in mosquitofish from a nearby reference site. Mosquitofish from Kesterson typically contained geometric mean concentrations greater than 100 mg Se/kg, in comparison to 1 to 2 mg Se/kg at the reference site. In 1984, one composite sample from Kesterson contained 430 mg Se/kg, and the means for fish from two of the ponds exceeded 300 mg/kg.

Laboratory bioaccumulation studies discussed above describe bioaccumulation of selenium by fish in relation to different waterborne forms of selenium and illustrate the importance of dietary exposure as a route of selenium uptake by fish. Studies report more frequently the rates of uptake of different forms of selenium than depuration after exposure to uncontaminated media (food and water). In one study,[80] cycling and fate of selenite and selenomethionine were compared in a 318-day experiment in which the selenium was introduced as an acute release into two experimental ponds. Biotic components sampled included periphyton, rooted plants (*Elodea canadensis*), snails (*Helisoma* sp.), and mosquitofish. Uptake rates for selenomethionine in the biota were at least an order of magnitude greater than for selenite, but elimination rates were similar (different by less than a factor of two). This study also demonstrated the importance of sediment and organic detritus in the recycling/remobilization of selenium within the ponds.

17.3.3.2 Marine

Estuarine and marine fish tend to have higher selenium concentrations than do freshwater species, but the differences for most species are generally less than an order of magnitude and sometimes are very similar.[19] Higher concentrations were found in species such as tunas and marlins. Selenium has been shown to accumulate with age/length in several studies of marine fish, and a positive correlation between selenium and mercury has been demonstrated in several species.[109] A positive correlation of selenium with mercury in fish may be a significant factor influencing bioaccumulation. One study has shown that a lower portion of selenium is present as selenate in marine fish (24%) than in freshwater fish (36%).[110]

Kasegalik Lake (on the Belcher Islands, Hudson Bay, in the southern Canadian Arctic) is known to have both salt- and freshwater populations of Arctic char (*Salvelinus alpinus*).[111] Fish of both types were analyzed for mercury and selenium. There was an apparent difference in the two populations for mercury concentrations, but there was no difference between salt- and freshwater groups for selenium (which varied from 0.7 to 1.9 mg Se/kg, wet weight). Thus, some of the apparent differences in selenium levels between marine and freshwater fish may be attributed to species differences as well as relationships between selenium and mercury in larger, long-lived marine species.

17.3.4 Amphibians and Reptiles

Background concentrations in livers of amphibians and reptiles are 2.9 to 3.6 mg/kg and in other tissues 1 to 3 mg/kg.[17] Many fewer studies of selenium bioaccumulation in amphibians and

reptiles have been conducted than those of other animals,[7,17,19] but information on bioaccumulation is summarized below.

17.3.4.1 Amphibians

Selenium concentrations in adult southern toads (*Bufo terrestris*) from coal-ash-settling basins (17.4 mg/kg, whole-body analysis) were elevated by comparison to toads from a reference site (2.10 mg/kg).[112] When other toads were transferred from the reference site to the settling basin for a period of 7 weeks, their concentrations increased significantly — from 2.10 mg Se/kg to 5.46 mg/kg. Sediments in the ash basin contained 4.4 mg Se/kg, whereas those at the reference site had 0.10 mg Se/kg. Frog (*Rana clamitans*) tadpoles from a fly-ash-contaminated pond contained 4.7 mg Se/kg (wet weight), whereas those from a reference site had 1.5 mg/kg.[75] Red-spotted newts (*Notophthalmus viridescens*) from those same sites contained 4.2 and 1.8 mg Se/kg (wet weight), respectively. Similarly, bullfrog tadpoles (*Rana catesbeiana*) from an ash-waste site had elevated selenium (25.7 mg/kg) by comparison to a reference site (3.4 mg/kg).[113]

Because tadpoles apparently accumulate metals readily from sediments and vegetation they consume, some authors (e.g., References 114, 115) have suggested that they may be good indicators of contaminated environments. To evaluate the effects of gut contents (especially ingested sediment) on analyses of bullfrog tadpoles, wild-caught tadpoles were maintained in clean water and analyzed after 0, 24, 48, and 72 h and then analyzed for metals to test the effects of "clearing" of the gut contents.[115] Metals concentrations in whole bodies and digestive tracts also were evaluated separately. Selenium concentrations in the bodies and tails of the tadpoles were unaffected by clearing, although selenium concentration was higher in the digestive tract than in the body without digestive tract, in the tail, or in the whole body. The tadpoles had higher selenium concentrations than those found in the sediment from the sampling location.

Bullfrogs collected from reference sites in the San Joaquin Valley, California contained 1.0 to 1.9 mg Se/kg when analyzed as whole-body samples.[116] Livers of three bullfrogs from other reference sites in the San Joaquin Valley contained 3.6 to 9.3 mg Se/kg (mean 6.2 mg/kg).[117] The lower end of that range is probably representative of background, whereas the upper value may reflect some elevation above background. Few frogs were observed in nearby Kesterson Reservoir, probably because of the highly saline water it contained. However, ten bullfrogs were collected from the San Luis Drain (which conveyed agricultural subsurface drainage waters to Kesterson); livers of these bullfrogs contained 25 to 88 mg Se/kg (mean 45 mg/kg). These liver concentrations were similar to the selenium concentrations in aquatic insects and mosquitofish in the Drain.

17.3.4.2 Reptiles

Livers of banded water snakes (*Nerodia fasciata*) from ash-settling basins associated with a coal-burning electric power plant were analyzed for selenium and several other elements that were found at elevated levels in sediment within the basins.[118] Selenium concentrations in the livers of snakes from these basins averaged about 140 mg/kg, compared to 3.62 mg Se/kg in livers of snakes from a reference site. Water snakes were found to be feeding on small bullfrogs and bullfrog tadpoles, green treefrogs (*Hyla cinerea*), southern toads, bluegill sunfish (*Lepomis macrochirus*), mosquitofish, red-fin pickerel (*Esox americanus*), and largemouth bass (*Micropterus salmoides*). Samples of those potential prey items were collected at the polluted ash-basin site and at the reference site. Mean selenium concentrations in the amphibians and fish from the polluted site were about 10 to 27 mg/kg, whereas those in amphibians and fish from the reference site were typically less than 2 mg/kg. Thus, the water snakes from the ash-basin site bioaccumulated selenium in their livers to concentrations about tenfold the prey diet concentrations, and those at the reference site had selenium concentrations that were about equal to the prey item levels. Selenium concentrations in livers showed a significant linear relationship with snake body mass.

Water snakes (*Nerodia* sp.) from Florida contained 0.3 to 0.5 mg Se/kg (whole body, wet weight).[119] Whole-body selenium concentrations in lizards and snakes from reference areas in the San Joaquin Valley averaged 0.7 to 2.0 mg/kg.[116] Livers of gopher snakes (*Pituophis melanoleucus*) from other reference sites in the San Joaquin Valley contained mean selenium concentrations of 2.05 and 2.14 mg/kg.[117] Livers of gopher snakes from the nearby Kesterson Reservoir had a mean selenium concentration of 11.1 mg/kg. Selenium bioaccumulation in these snakes at Kesterson and associated reference sites reflected selenium levels in prey species for the snakes at those sites.

Selenium levels in the plasma of wild and farm-reared American alligators (*Alligator mississippiensis*) reflected selenium concentrations in their diet.[120] Plasma selenium levels in captive alligators fed fish (*Micropogon undulatus*) were significantly higher (monthly means of 0.23 to 0.27 mg/kg) than in captive animals fed nutria (*Myocastor coypus*) (0.16 to 0.23 mg/kg) or in wild females (0.15 to 0.20 mg/kg); plasma selenium levels in the alligators fed nutria and in the wild females were not significantly different from each other. Fish contained 2.8 mg Se/kg (dry weight), compared to only 0.04 mg/kg (wet weight) in the nutria. Diet of the wild alligators was composed primarily (~70%) of nutria.

17.3.5 Birds

Background concentrations in whole-body birds are < 2 mg/kg.[17] However, bioaccumulation in birds is more frequently (and appropriately) evaluated on the basis of various tissues, as discussed below.

17.3.5.1 Eggs

Mean background concentrations in eggs of freshwater and terrestrial species are < 3 mg Se/kg (typically 1.5 to 2.5 mg/kg), and maximums are < 5 mg Se/kg.[17,19] In a wide variety of species, selenium concentrations (on a dry-weight basis) in bird eggs range from roughly equivalent to about three or four times the concentrations in the diet of the female at the time of egg-laying.[7,121–127]

When birds feed on selenium-contaminated diets during the laying season, the exposure is quickly reflected by elevated levels of selenium in eggs.[128] Similarly, when the birds are switched to a clean diet, selenium concentrations in eggs decline quickly. When mallard (*Anas platyrhynchos*) hens were fed a diet containing 15 mg Se/kg (as selenomethionine), levels peaked in eggs (to about 13 to 20 mg Se/kg, wet weight) in about 2 weeks on the treated diet and leveled off at a relatively low level (<5 mg Se/kg, wet weight) after about 10 days back on the untreated diet. The findings of this study have important implications for evaluation of field exposures, such as how quickly and for what duration selenium exposure may adversely affect bird reproduction. Concentrations of selenium in eggs are especially important because they provide the best samples for evaluation of potential adverse reproductive effects (see also later section).[129]

Selenium concentrations in the eggs of marine species are variable but may be higher, even in remote areas.[7] For example, eggs of three species (wedge-tailed shearwater [*Puffinus pacificus*], red-footed booby [*Sula sula*], and sooty tern [*Sterna fuscata*]) were sampled at four locations throughout the Hawaiian archipelago, from Oahu to Midway.[130] Mean concentrations (converted from wet weight) varied only slightly by location — from 4.4 to 5.3 mg Se/kg for shearwaters, 5.0 to 6.1 mg Se/kg for boobies, and 4.1 to 5.1 mg Se/kg for terns — but all were higher than typical of freshwater species. Surprisingly low selenium concentrations were found in eggs of white-winged scoters (*Melanitta fusca*) when compared to the concentrations of selenium found in their livers.[131]

Elevated selenium concentrations have been found in eggs of birds nesting in areas affected by agricultural drainage, coal-fly-ash disposal, and discharge from an oil refinery.[7,25,91,129,132,133] At Kesterson Reservoir, California, mean selenium concentrations in bird eggs during 1983 to 1985 were as high as 69.7 mg/kg (in eared grebes [*Podiceps nigricollis*]), with many of the species means exceeding 20 mg Se/kg.[91] Some success in reducing selenium exposures of aquatic birds at evap-

oration ponds for agricultural drainage water was achieved by placing freshwater mitigation ponds near the contaminated ponds.[134]

17.3.5.2 Livers

Background concentrations in bird livers are < 10 mg Se/kg.[17] In a manner similar to that for eggs, Heinz et al.[135] found that selenium concentrations in the liver respond quickly when birds are placed on or taken off a selenium-contaminated diet (see further description of this study below under Muscle). Thus, selenium concentrations measured in the livers of birds sampled outside the breeding season are not good predictors of potential reproductive effects. In laboratory studies of reproductive effects, livers of male mallards had higher concentrations of selenium than those of females, probably because females excreted part of the selenium they had accumulated through egg-laying.[121,122] Nevertheless, analysis of livers of either male or female field-collected birds can provide a useful indication of the relative level of exposure experienced by the population.

In laboratory studies with birds fed diets containing selenomethionine, selenium concentrations in livers of mallards, black-crowned night-herons (*Nycticorax nycticorax*), and eastern screech-owls (*Otus asio*) ranged from roughly equal to the dietary concentration to about three times the dietary level.[121–126]

At Kesterson Reservoir and a nearby reference site, several species of aquatic birds were sampled early and late in the nesting season as adults, and juveniles were sampled at about the same time as the late-season adults.[136] Selenium concentrations in livers of these birds were consistently much higher at Kesterson than at the reference site for both early and late samplings and seemed to reflect the period of exposure as well as the foraging range of the birds at the two sites. For example, adults of the more mobile species (such as dabbling ducks, *Anas* spp.) showed only three- to fivefold differences in mean selenium concentrations between sites, whereas more sedentary species (such as American coot [*Fulica americana*] and black-necked stilt [*Himantopus mexicanus*]) typically showed 10- to 20-fold differences. Selenium concentrations in juvenile birds were generally similar to those in late-season adults.

Selenium concentrations in the livers of aquatic birds from a number of other areas receiving subsurface agricultural drainage also were elevated and have reflected periods of residence in the contaminated areas.[25,137–139] When use of subsurface agricultural drainage waters for wetland management was curtailed and replaced with better-quality water, selenium concentrations in aquatic birds declined.

Livers of diving ducks (such as scoters [*Melanitta* spp.] and scaups [*Aythya* spp.]) from estuarine habitats have been found to contain higher concentrations of selenium than other aquatic birds in the same habitats.[140–143] The apparent reason for the higher concentrations of selenium in the diving ducks is that they forage on benthic organisms, which bioaccumulate selenium to a higher degree than foods of some of the other aquatic birds.

17.3.5.3 Kidneys

Selenium concentrations in kidneys of birds from selenium-normal areas were somewhat higher than those in the liver (liver/kidney ratios of less than L), but concentrations in the two tissues were similar in birds from selenium-contaminated Kesterson Reservoir and in the Imperial Valley of California.[136,144,145] Selenium concentrations in American coots from Kesterson Reservoir and the reference site were significantly correlated ($r = 0.98$). Significant ratios and significant positive correlations also have been found in other field studies (e.g., Reference 146).

17.3.5.4 Muscle

Background selenium concentrations in muscle tissues of birds are 1 to 3 mg/kg.[17] Concentrations increase and decrease in response to changes in dietary exposure, but the changes occur more

slowly than those in eggs or liver.[135] However, for muscle as well as other tissues, when tissue levels of selenium are made very high by elevated dietary exposures and the birds are then placed on a lower-selenium diet, the loss rate for all tissues is fast at first but then slows down.

Mallards were placed on a diet containing 10 mg Se/kg for 6 weeks; predicted equilibrium time for concentrations in the breast muscle of females (81 days) was more than ten times that for time to equilibrium in the liver (7.8 days).[135] The ducks were then provided an untreated diet for 6 weeks, and loss of selenium from liver and muscle was monitored; half-times were 18.7 days for liver and 30.1 days for muscle. Males reached similar levels of selenium in the liver and breast muscle as females and declined to similar levels when treatment ended. In another experiment with mallards, females were fed increasing levels of selenium until some died; survivors were then switched to an untreated diet and selenium was measured in blood, liver, and breast muscle over 64 days. Half-times were 9.8 days for blood and 23.9 days for muscle. Selenium initially decreased in liver by one-half in 3.3 days, with subsequent half-times of 3.9, 6.0, and 45.1 days.

Selenium concentrations in breast muscle from juvenile ducks (*Anas* sp.) at Kesterson Reservoir and a reference site were measured because of concern about human consumption of ducks harvested in the vicinity of Kesterson.[136] Mean selenium concentrations were higher at Kesterson than the reference site and were only slightly lower than those in livers of these birds. However, the relationships between muscle and liver ($r^2 = 0.69$) of the ducks were considerably more variable than those between kidneys and livers of American coots from the two sites ($r^2 = 0.97$).

17.3.5.5 Blood

Background concentrations in whole blood are 0.1 to 0.4 mg Se/kg on a wet-weight basis.[17] In experimental studies, selenium concentrations in blood of mallards and American kestrels (*Falco sparverius*) reflected dietary exposure levels.[127,135,147–149] In kestrels, maximal blood concentrations were about the same as those in the selenomethionine-supplemented diet. Mallards receiving selenium (as selenomethionine) at dietary concentrations of 10, 25, or 60 mg/kg had blood-selenium concentrations of 4.5, 8.9, or 16 mg/L (wet-weight basis).[148] The concentration of selenium in blood increased in a time- and dose-dependent manner and reached a plateau after 40 days. (The study by Heinz et al.[135] is described in the previous section.)

Selenium concentrations were measured in terrestrial birds of several species from Kesterson Reservoir, the area surrounding that site, and several reference areas in California from 1994 to 1998.[150] Except for loggerhead shrikes (*Lanius ludovicianus*), blood-selenium was higher in birds from within Kesterson than in birds from other areas. For shrikes, the mean concentrations for birds from Kesterson (13 mg Se/kg [dry weight]) were not significantly different than those from nearby surrounding areas (8.5 mg Se/kg), although the maximum selenium concentration at Kesterson (38 mg/kg) was more than twice the maximum for the surrounding area (16 mg/kg). Among species at Kesterson Reservoir, blood-selenium was higher in loggerhead shrikes and northern harriers (*Circus cyaneus*) than in the other species (hawks and owls) sampled.

17.3.5.6 Feathers

Background concentrations of selenium in feathers are 1 to 4 mg/kg and are typically less than 2 mg/kg.[17] Selenium concentrations may be higher in the feathers of birds from areas with elevated levels of mercury because of the interactions between these two elements.

Analysis of feathers may provide useful information concerning exposures of birds to selenium if they are considered carefully. It is important to recognize that the selenium may have been deposited into the feathers at the time they were formed (which may have been months earlier and thousands of miles away from the sampling time and location), or the selenium may be the result of external contamination.[151–154] Concentrations also may have been reduced through leaching. Different kinds of feathers from the same bird may contain different concentrations, depending partly on when and where the feathers were grown during the moult cycle.

Feathers of five species of seabirds were sampled at Johnston Atoll (about 1300 km southwest of Hawaii) and three species at Manana Island, Oahu, Hawaii, and analyzed for selenium and several other elements.[155] Selenium concentrations were generally higher in brown noddies (*Anous stolidus*) than in other species at both locations, and noddies had significantly higher concentrations at Johnston Atoll (11.1 mg Se/kg) than Hawaii (7.59 mg Se/kg).

17.3.6 Mammals

Background whole-body selenium concentrations in terrestrial and freshwater mammals are < 1 to 4 mg/kg.[17] In muscle, concentrations are typically < 1 mg Se/kg; in liver, they range from about 1 to 10 mg Se/kg and are typically ≤5 mg/kg; in hair, normal concentrations are <3 mg/kg in individual samples, with population averages ranging from 0.5 to 1.5 mg Se/kg; selenium in whole blood is typically 0.1 to 0.5 mg/L, with concentrations below 0.1 mg/L indicating a deficiency in domestic livestock. Both hair and blood are considered good media for sampling/monitoring selenium status of live mammals.

17.3.6.1 Aquatic/Marine

Selenium concentrations in raccoons (*Procyon lotor*) collected at Kesterson Reservoir, California averaged 19.9 mg/kg in liver, 28.3 mg/kg in hair, 21.6 mg/kg in feces, and 2.61 mg/L in blood (wet weight in blood, others in dry weight).[156] These concentrations were 12, 30, 21, and 10 times higher, respectively, than those found in raccoons from a nearby reference site. Selenium concentrations in hair provided the strongest statistical separation between the two study areas. Selenium concentrations in raccoons and river otters (*Lontra canadensis*) at sites in Canada were similar to each other, but they were higher than those found in beavers (*Castor canadensis*).[157]

Selenium concentrations in the tissues of marine mammals are highly variable, with some animals having concentrations in the liver or other tissues that are typical of mammals in freshwater or terrestrial environments and others (even of the same species in the same areas) having greatly elevated levels.[19,109] Part of this variation may be due to interactions between selenium and mercury, with much greater bioaccumulation (especially as reflected by concentrations in the liver) when the animals are exposed to elevated levels of mercury.

Selenium concentrations greater than 100 mg/kg have been reported for livers of pilot whales (*Globicephala macrorhynchus*),[158] beluga whales (*Delphinapterus leucus*),[159] striped dolphins (*Stenella coeruleoalba*),[160] bottle-nosed dolphins (*Tursiops truncatus*),[161] ringed seals (*Phoca hispida*),[162] harbor seals (*P. vitulina*),[163] gray seals (*Halichoerus grypus*),[164] and California sea lions (*Zalophus californianus*).[165]

17.3.6.2 Terrestrial

Mean selenium concentrations in several species of small mammals at Kesterson Reservoir, California, were greatly elevated by comparison to a nearby reference area.[166] For example, means for California voles (*Microtus californicus*) from Kesterson were as much as 522 times those from the reference area (means up to 119 mg Se/kg vs. 0.228 mg Se/kg in livers). There were species-to-species differences at Kesterson; higher selenium concentrations occurred in carnivorous species and species that fed on foods closely linked to the pond water. There were also pond-to-pond differences at Kesterson, with mammals reflecting a pattern similar to that for aquatic organisms (described above).

Following the conversion of Kesterson Reservoir to a terrestrial habitat in 1988 (as described above), selenium concentrations in small mammals have been monitored periodically.[89,90] Mean whole-body selenium concentrations in mice on a site-wide basis are typically 5 to 7 mg Se/kg and tend to be lower in voles than in mice. However, the selenium levels tend to vary spatially

within the site, especially as related to habitat type and history of management activities that have altered the site since it served as a reservoir for agricultural drainage waters.

Elevated levels of selenium were found in woodchucks (*Marmota monax*) from the vicinity of a fly-ash landfill,[167] and in American bison (*Bison bison*), elk (*Cervus elaphus*), and mule deer (*Odocoileus hemionus*) that ate forage with elevated levels of selenium in Wyoming.[168] In contrast, analysis of blood from free-ranging mule deer sampled from a number of herds in California indicated the deer were selenium-deficient.[169–173] Several methods of selenium supplementation to the deer were tested; some were more practical and effective than others and resulted in increased reproductive performance of the herds.

17.4 EFFECTS IN ANIMALS

17.4.1 Nutritional Requirements vs. Toxicity

Selenium is essential for animal nutrition at sub-mg/kg dietary concentrations, but it is toxic to sensitive species when concentrations are only a few mg/kg. For most animals, dietary requirements appear to fall in the range between about 0.05 and 0.5 mg/kg.[5,19,46,56,174–179] Thresholds for toxicity are usually not much more than an order of magnitude higher than the concentrations that cause deficiency (as described below). Selenium deficiency is a more widespread problem for animal health than is excess selenium, so the issue of selenium ecotoxicology is one of balance between meeting the dietary requirements without exceeding toxicity thresholds. Signs of selenium deficiency have been reported in many domesticated species (including chickens, turkeys, pheasants, quail, ducks, cattle, horses, goats, sheep, and swine) as well as wild species (including species such as fish, bighorn sheep [*Ovis canadensis*], pronghorns [*Antilocapra americana*], elk, and deer). In poultry, selenium deficiency causes severe pancreatic atrophy as well as reduced egg production and hatchability; in mammals it causes poor health and reproduction. One of the more common myopathies resulting from selenium deficiency (referred to as "white muscle disease") has been reported in at least 30 of the 50 U.S. states.

Selenium deficiency in some species can be ameliorated through a variety of means including addition of selenium to diets of domestic species, use of intrarumenal boluses that release selenium slowly, and application of selenium-containing fertilizers to grazing lands.[5,176–179] Selenium fertilization and use of intrarumenal boluses have been tested for improvement of reproduction in free-ranging mule deer, sometimes with significant benefit.[170,172,173] However, selenium deficiencies in other kinds of wildlife may not be corrected as easily as those in species such as deer.

17.4.2 Toxic Effects

Selenosis and selenium toxicosis are used interchangeably to refer to the pathological conditions resulting from ingestion of high-selenium diets. Those conditions include impaired health and reproduction, as discussed below in relation to vertebrate animals. When water is the only exposure route, selenium is not very toxic to fish or wildlife.[17,20,57] Eggs and larvae of fish and amphibians may be the most sensitive life stages of vertebrate animals to direct effects of waterborne selenium. Eggs and larvae of fish and birds are very sensitive to the lethal or teratogenic effects of selenium transferred to the eggs by the female parent (as discussed below).

17.4.2.1 Invertebrates

Invertebrates are not particularly sensitive to the toxic effects of selenium, and there is limited information concerning adverse effects to invertebrates under field conditions.[17] In laboratory studies, selenite was found to be more toxic than selenate, regardless of whether the effects were

measured as direct toxicity or impairment of reproduction.[20,180,181] Duration of exposure is also an important factor, with toxicity increasing as exposure time increases, at least up to 14 days. For example, the 96-hour LC_{50} for the scud (*Hyalella azteca*) was 340 µg Se/L or 760 µg Se/L in different studies,[182,183] whereas the 14-day LC_{50} was only 70 µg Se/L.[184] Similarly, for the *Daphnia magna* the 96-hour LC_{50} was 710 µg Se/L and the 14-day LC_{50} was 430 µg Se/L.[184]

In a comparison involving the cladoceran *Daphnia magna* and larvae of the midge *Chironomus riparius*, the daphnids were more acutely sensitive than midges to the toxic effects of inorganic selenium.[185] An organic form of selenium (selenomethionine) was toxic to daphnids but relatively nontoxic to midges. Tissue concentrations of 14.7 and 31.7 mg Se/kg in daphnids were associated with reduced growth and reproduction, respectively. Nevertheless, these invertebrates were not adversely affected at waterborne concentrations that would affect fish or would bioaccumulate in the food chain to levels that are harmful to fish or aquatic birds.

In evaluating the potential for selenium to cause toxicity to aquatic invertebrates, it is important to note that other elements can affect the toxicity of selenium. For example, sulfate can reduce the bioavailability and toxicity of selenate selenium (the predominant form in many water bodies) to invertebrates.[99]

Biologically incorporated selenium (presumably in organic forms) in widgeongrass from agricultural drainwater evaporation ponds in the San Joaquin Valley, California, caused significant reductions in growth of midge (*Chironomus decorus*) larvae that were cultured on detrital material from these plants.[79] Significant reductions in growth were observed in midges exposed to plant substrate having more than about 10 mg Se/kg, and relative bioaccumulation (as BAF) was inversely related to selenium concentrations in the plant material (i.e., a relatively smaller amount was taken up when concentrations were greater than 20 mg Se/kg).

17.4.2.2 Fish

Several recent publications provide reviews of the effects of selenium in fish and describe case studies of such effects.[12,14,17,19,23,25,186,187] Those references provide more detailed descriptions of tissue or exposure media concentrations that are associated with no effects or with adverse effects in fish. Some also emphasize that a combination of biological (e.g., condition of larvae/fry, reproductive success, etc.) and chemical (e.g., analysis of ovaries/eggs, whole-body fish, or food-chain organisms) indicators should be used in evaluating risks to fish populations. The mortality of larvae/fry that is associated with excess selenium can have important effects on populations, resulting in lack of recruitment and subsequent declines or disappearance of affected species in a contaminated water body without evidence of "die-offs." Given enough time, once the levels of selenium input are reduced, populations may recover; however, contaminated sediments will serve as a continuing source for selenium cycling.[188]

Excess selenium in the diet of fish leads to substitution of selenium for sulfur during protein synthesis, among other effects.[23] Substitution of selenium for sulfur disrupts normal chemical bonding, resulting in improperly formed and dysfunctional proteins or enzymes. This causes a variety of toxic effects at subcellular, cellular, organ, and system levels.[103,189] These effects are exhibited through effects on reproduction (especially in the form of teratogenesis) and reduced survival of young fish as well as effects on health, physiology, and survival of older fish.

There is general agreement that early life stages (i.e., eggs, larvae, and fry) are more sensitive to the adverse effects of excess selenium than are adult fish, and that some species of fish are more sensitive than others.[14,17,23,186,190] Selenium concentrations in the ovary/eggs as well as those in whole-body fish and the diet are most useful in evaluating risks of adverse effects (i.e., more useful than selenium concentrations in water or sediment, or in livers or other tissues). However, there is no uniform agreement on the selenium concentrations that represent thresholds for adverse effects.

Selenium concentrations in ovaries/eggs are considered to represent a threat of adverse effects when they are greater than 10 mg/kg,[14,23,190] 7 to 13 mg/kg,[17] or 17 mg/kg.[186] At concentrations

exceeding the threshold, there is a large increase in the occurrence of teratogenic deformities and a concomitant reduction in survival of the larvae/fry. Although many of the larvae/fry die as a result of the deformities, some fish may survive and have noticeable deformities as adults. Selenium-related deformities observed in fish larvae and fry are due to their absorbing/using the yolk from the egg, which contains elevated levels of selenium deposited there by the female parent for their nourishment and development. Feeding excess but sublethal levels of selenium to fry, juvenile fish, or adult fish does not cause deformities such as those caused through maternal transfer of selenium to the egg.[191–193]

Examples of the kinds of teratogenic deformities that are caused by selenium are provided by Lemly.[23] The typical deformities include lordosis (concave curvature of the lumbar region of the spine), scoliosis (lateral curvature of the spine), kyphosis (convex curvature of the thoracic region of the spine, resulting in a "humpback" condition), missing or deformed fins, missing or deformed gills and gill covers (opercle), abnormally shaped head, missing or deformed eyes, and deformed mouth.

Selenium concentrations in whole-body fish are considered to represent a threat of adverse effects to health or reproductive success when they are greater than 4 mg/kg[14, 190] or greater than 4 to 6 mg/kg;[17] however, DeForest et al.[186] evaluated results of available studies and considered the threshold to be 6 mg/kg for cold-water and anadromous species and 9 mg/kg for warm-water fish. These differences in values result from different interpretations of the available information from the various studies. Despite the differences, however, it is important to note that whole-body selenium concentrations in the range of 4 to 9 mg/kg are recognized to reflect risk of adverse effects in fish.

Threshold selenium concentrations in the diet of fish that are associated with adverse effects have been estimated as 3 mg/kg,[14, 190] as 3–8 mg/kg,[17] or separately as 11 mg/kg for cold-water and anadromous species and 10 mg/kg for warm-water fish.[186] As described in the previous section (Bioaccumulation), different forms of selenium bioaccumulate at very different rates in food-chain organisms, with selenomethionine > selenite > selenate. These differences in bioaccumulation can greatly affect the degree of risk associated with particular waterborne selenium concentrations. However, studies indicate that once the selenium is biologically incorporated in the food organisms, the risk to fish is similar at equivalent dietary concentrations.

In waterborne exposures, organic selenium (selenomethionine) also is much more toxic than selenite, which is more toxic than selenate. For example, the 96-hour LC_{50} values for bluegill (13 µg Se/L) and striped bass (*Morone saxatilis*; 4 µg Se/L) based on selenomethionine were only a small fraction of the LC_{50}s for selenite (7800 to 13,000 and 1000 µg Se/L, respectively) and selenate (98,000 and 39,000 µg Se/L).[181] However, as duration of exposure increases, the toxicity of selenium increases. For example, when selenium was introduced to outdoor experimental streams at 10 or 30 µg/L, all adult bluegill sunfish at the higher exposure level died in less than a year, and there also was significantly reduced survival of those at 10 µg Se/L when compared to controls.[194] Exposure of adults at both 10 and 30 µg Se/L for 40 weeks before spawning resulted in reduced embryo and larval survival and produced larvae with a high incidence of edema, lordosis, and internal hemorrhaging.

17.4.2.3 Amphibians and Reptiles

There is only limited information about the toxic effects of selenium in amphibians, and there is even less information about its effects in reptiles.[17,19,195–198]

In laboratory exposures, amphibian embryos and tadpoles were about as sensitive as aquatic invertebrates and fish larvae/fry to the effects of waterborne inorganic selenium.[199–201] In short-term toxicity tests (typically 96-h exposures, but up to 3 days) with the South African clawed frog (*Xenopus laevis*), LC_{50} values for both selenite and selenate were 1500 µg Se/L or greater. Not surprisingly, the LC_{50} values decrease as exposure duration increases. The 7-day LC_{50} for this species was 456 µg Se/L, but in a 7-day exposure with narrow-mouthed toad (*Gastrophryne carolinensis*)

embryos, the LC_{50} values was only 90 µg Se/L.[202, 203] The low value for the narrow-mouthed toad apparently reflects species differences in sensitivity or perhaps differences in exposure conditions.

Adult and larval amphibians exposed to trace elements in coal combustion waste ponds showed physiological and morphological changes as well as bioaccumulation of some elements.[112,113] For example, tadpoles of the bullfrog collected in a coal-ash basin and a downstream drainage swamp had a reduced number of labial teeth and deformities of labial papillae when compared with tadpoles from reference areas. Tadpoles with deformities were less able to graze periphyton than were normal tadpoles, and they had lower growth rates. These tadpoles had significantly elevated concentrations of selenium (25.7 mg/kg) by comparison to a reference site (3.37 mg/kg). They also had significantly greater concentrations of arsenic, barium, cadmium, and chromium than those from the reference site, but they did not significantly bioaccumulate copper or lead in comparison to the reference site. Thus, it is not possible to determine which elements caused the effects.

Banded water snakes from a site contaminated by coal combustion wastes had high concentrations of trace elements, especially selenium and arsenic but also cadmium, chromium, and copper, by comparison to snakes from a nearby reference site.[118] (Results of analyses of livers and potential prey items were summarized in a previous section.) Snakes from the ash-basin site exhibited mean standard metabolic rates 32% higher than snakes from the reference site. The authors concluded that snakes from the contaminated site probably allocate more of their energy to maintenance and theoretically should have less energy available for growth, reproduction, and storage.

17.4.2.4 Birds

Selenium has been identified as the agent responsible for mortality and reproductive failure in birds at a number of sites in the United States (see USDI[17] and Skorupa[25] for a partial list). Much of the research evaluating the effects of selenium in birds has been related primarily to discharges of subsurface agricultural drainage water,[7,129] but effect levels in bird diets and eggs probably are similar once the selenium has been accumulated by food-chain organisms.

Selenium became recognized as a significant environmental contaminant for wildlife in 1983, with the discovery of developmental abnormalities and excessive embryonic and adult mortality in aquatic birds at Kesterson Reservoir, California.[7,91,132,144,204,205] Observations in the field were corroborated through a series of laboratory studies that documented the association between excessive dietary selenium and effects on reproduction and young birds as well as adult health, physiology, and survival. Those effects are described in several other reviews (e.g., References 4, 7, 8, 13, 17, 19, 25) and are summarized below.

17.4.2.4.1 Reproduction and Young Birds

Prior to the 1980s, most studies of avian reproductive effects in relation to selenium were conducted with domestic poultry.[4,7,13] Studies of wild birds to determine the effects of selenium on reproduction and other aspects of avian biology have been conducted in the field and in the laboratory since 1983. Reproductive effects of selenium reported in wild aquatic birds and domestic poultry include embryo mortality and teratogenesis as well as the failure of adult birds to nest.

Excess selenium in the diet of female birds during the period just before egg-laying results in transfer of selenium to the eggs at harmful levels, although the sensitivity varies among species.[7,8,13,25,128,129,206] Characteristic effects observed in the field and laboratory studies include reduced hatchability of eggs (due to embryo mortality) and high incidence of developmental abnormalities (due to teratogenesis). Selenium-induced abnormalities are often multiple and include defects of the eyes (microphthalmia and possible anophthalmia [i.e., abnormally small or missing eyes]), feet or legs (amelia and ectrodactylia [absence of legs or toes]), beak (incomplete development of the lower beak, spatulate narrowing of the upper beak), brain (hydrocephaly and exencephaly [fluid accumulation in the brain and exposure of the brain]), and abdomen (gastroschisis [an open fissure

of the abdomen]). Most of these abnormalities are illustrated through photographs that have been published elsewhere (e.g., References 4, 7, 8, 132, 144).

Reproductive effects have been produced in laboratory dietary studies using both organic and inorganic forms of selenium (e.g., in mallards and domestic chickens [*Gallus gallus*]).[4,121,122,124,125,207–210] Selenomethionine, the most bioavailable and toxic of the different selenium compounds studied, is associated with reduced reproductive effects in mallards at dietary concentrations as low as 7 mg Se/kg.[125] This form of dietary selenium has been found to be the best surrogate for use in feeding studies to parallel the dose-effect levels found in field studies with birds.

Studies at Kesterson Reservoir showed there was poor hatching success of fertile eggs of a number of aquatic bird species[7,204] and that there was also poor survival of those young that did hatch.[7,211] This was attributed to high levels of selenium in the diet of the breeding females as well as the diet of newly hatched birds. Most aquatic insects at Kesterson had mean selenium concentrations greater than 100 mg/kg, and some composite samples exceeded 300 mg/kg.[40–42] Mallard ducklings were fed diets containing either sodium selenite or selenomethionine at concentrations of 0, 10, 20, 40, or 80 mg Se/kg from hatching to 6 weeks of age.[212] Among ducklings that received selenomethionine, 100% of the group receiving 80 mg Se/kg died in less than 6 weeks, and 12.5% of those that received 40 mg Se/kg died during the exposure period.

Predictive criteria for avian selenosis were developed through a broad-scale program that collected response data for avian teratogenesis at selenium-impacted and reference aquatic sites in the San Joaquin Valley, California.[17,206] The largest cumulative sampling effort occurred within the Tulare Basin area in the southern San Joaquin Valley. In the Tulare Basin, evaporative disposal of subsurface irrigation drainage water was accomplished by letting it evaporate from 25 shallow impoundments. Even though the impoundments were not constructed to attract wildlife and were devoid of emergent vegetation (i.e., cattails, bulrush, etc.), large populations of nesting waterbirds used the sites. Two of the most abundant waterbirds that nested at the evaporation basins were American avocets (*Recurvirostra americana*) and black-necked stilts (*Himantopus mexicanus*). Water discharged to the evaporation basins contained from < 1 to > 1000 μg Se/L; consequently, selenium concentrations in eggs from this region spanned four orders of magnitude (< 1 to > 100 mg/kg egg selenium).

Using data on selenium in eggs from the Tulare Basin, combined with data from several other western sites where elevated selenium was found, a detailed exposure-response relationship was documented.[17,25,206] Statistically distinct teratogenesis response functions were delineated for ducks, stilts, and avocets using the Tulare Basin data. The Tulare curves were used to estimate expected frequencies of teratogenesis for ducks, stilts, and avocets using other sites, and the predicted levels were tested against the observed frequencies from the sites. The predicted and observed frequencies of teratogenesis were not significantly different, and therefore the data were combined to generate final response curves. Using this data set, Skorupa[206] developed species-specific response curves for stilts and avocets and a composite duck curve (using combined data from gadwalls [*Anas strepera*], mallards, pintails [*A. acuta*], and redheads [*Aythya americana*]).

Based on the response coefficients and their standard errors, the teratogenesis function for ducks, stilts, and avocets were significantly different.[206] Within this data set, these responses represent "sensitive" (duck), "average" (stilt), and "tolerant" (avocet) species. The probability of overt teratogenesis in stilts increases markedly when selenium concentrations are greater than 40 mg/kg, with an EC_{10} for teratogenic effects of 37 mg/kg. In contrast, the threshold for teratogenesis (expressed as an EC_{10}) in mallards is 23 mg/kg, and in avocets it is 74 mg/kg.

A more sensitive measure of avian selenosis is reduced egg hatchability due to embryo inviability.[17,25,206] Egg selenium concentrations that cause embryo inviability are usually below the levels that cause embryo deformities. The threshold for mean egg selenium associated with impaired egg hatchability at the population level for black-necked stilts (and, therefore, the embryotoxicity threshold) was estimated to be 6 to 7 mg/kg. This threshold is approximately equivalent to the EC_{10} on a clutchwise (or henwise) basis and the EC_{03} on an eggwise basis.[33] Skorupa[206] used more than

400 sample eggs from black-necked stilt nests monitored at Tulare Basin, Kesterson Reservoir, and Salton Sea, California to identify the background rate of inviable stilt eggs (8.9%) and developed the following equation to determine the number of clutches containing at least one inviable embryo:

$$Y = \exp(-2.327 + 0.0503X)/(1 + \exp(-2.327 + 0.0503X)); r^2 = 0.18 \quad (17.1)$$

where Y is the probability of ≥ 1 inviable egg in a sampled clutch based on selenium concentration in a random sample egg (X). In contrast to the stilt, with a threshold of 6 to 7 mg Se/kg for effects on egg hatchability, the avocet is much less sensitive, with no effects on hatchability at concentrations below 60 mg Se/kg.

In his review of the available information concerning reproductive effects of selenium in birds, Heinz[13] suggested 10 mg Se/kg (converted from 3 mg Se/kg wet-weight basis he reported) in avian eggs as a threshold for effects on hatchability. Conclusions concerning egg-selenium threshold levels for effects in waterfowl depend on which data are included in the analyses, how the effects are expressed, and statistical approaches used in the analyses. For example, Fairbrother et al.[32,34] used some of the data available from experimental studies with mallards and concluded that the threshold for reduced hatchability (EC_{10}) is 16 mg Se/kg in the egg. However, Skorupa[33] disagreed with this conclusion.

The data available from six studies in which mallards were fed selenomethionine[121,122,124,125,207,208] are reanalyzed here using a two-parameter logistic regression model to calculate various effect levels for selenium, as follows: EC_{10} = 12.5 mg/kg; EC_{20} = 16.3 mg/kg; EC_{50} = 25.7 mg/kg in the eggs; in the diet, EC_{10} = 4.87 mg/kg; EC_{20} = 5.86 mg/kg; and EC_{50} = 8.05 mg/kg. Table 17.1 provides a brief summary of the relevant studies, including the dietary treatment level of selenium, reported egg hatchability, and mean selenium concentrations in the eggs. In addition, the table provides the egg hatchability results for the treatment groups as a proportion of the controls (to

Table 17.1 Effects of Dietary Selenium Exposure (as selenomethionine) on Egg Hatchability in Mallards and Associated Selenium Concentrations (mg/kg, dry weight) in Eggs

Dietary Selenium (mg/kg)[a]	N	Egg Hatchability (%)[b]	Hatchability as Proportion of Control	Egg Selenium (mg/kg)[c]	Reference
Control	11	65.7	1.0	0.16	121
10	5	30.9	0.470	15.2	
Control	32	59.6	1.0	0.59	122
1	15	70.7	1.19	2.74	
2	15	60.0	1.01	5.28	
4	15	53.4	0.896	11.2	
8	15	36.9	0.619	36.3	
16	9	2.2*	0.037	59.4	
Control	10	41.3	1.0	1.35	207
10 DL[d]	15	7.6*	0.184	30.4	
10 L[e]	15	6.4*	0.155	29.4	
Control	12	44.2	1.0	1.16	208
10	11	24.0	0.543	25.1	
Control	37	88.2	1.0	1.4	124
10	35	20.0*	0.227	37	
Control	33	62.0	1.0	0.89	125
3.5	29	61.0	0.984	11.6	
7.0	34	41.0*	0.661	23.4	

[a] Presented as nominal concentration in diet. In some (but not all) studies, control and treated diets were analyzed. Control diets typically contained 0.4 mg/kg selenium.
[b] Asterisks indicate hatchability significantly different than controls.
[c] When mean selenium concentrations in eggs were reported on wet-weight basis, concentrations were converted to approximate dry-weight basis, assuming 70% moisture.
[d] Seleno-DL-methionine.
[e] Seleno-L-methionine.

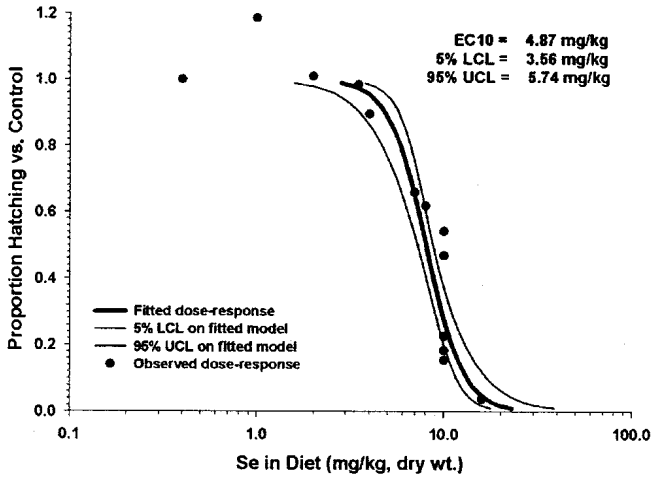

Figure 17.1 Mallard egg hatchability vs. control as a function of selenium concentration in diet.

take into account the variable hatching success for controls in the various studies). The results of these studies are illustrated graphically in Figures 17.1 and 17.2.

Fertility has been measured in only a small number of selenium studies. In American kestrels fed selenomethionine at 12 mg Se/kg, egg fertility was significantly reduced (by over 14%) compared to kestrels fed 6 mg/kg selenium.[127] Lack of reporting on fertility effects may be due in part to a general practice of simply including infertile eggs as inviable eggs in studies of selenium effects in birds (i.e., "infertility" effects may not be separated from "embryotoxic" effects in the overall measurement of hatchability). Failure to measure infertility as a separate endpoint may be due to the difficulty often associated with distinguishing infertile eggs from those containing embryos that have died very early in development. Nevertheless, decreased fertility is a distinct effect from embryotoxicity, particularly in that it indicates a mechanism acting on adult, rather than embryonic, physiology. Results obtained in kestrels suggest infertility may be a potentially important factor contributing to the overall reproductive impairment in some species. However, in mallards and black-crowned night-herons fed 10 mg Se/kg as selenomethionine, egg fertility was not reduced compared with controls.[121,123,207,208] Similarly, fertility was not affected in mallards fed diets containing selenium at 7 mg/kg[125] or 16 mg/kg[122] as selenomethionine, but hatchability of fertile eggs

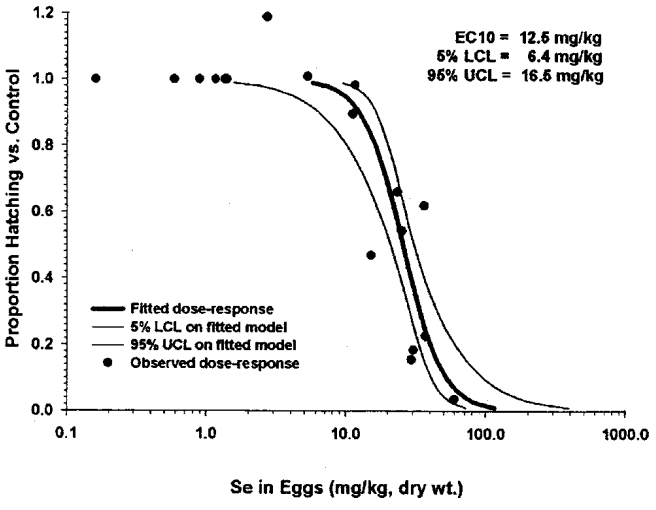

Figure 17.2 Mallard egg hatchability vs. control as a function of selenium concentration in eggs.

was significantly reduced. Thus, effects on egg fertility in mallards and night-herons are not likely to be as ecologically significant as reduced hatchability.

17.4.2.4.2 Adult Health, Physiology, and Survival

O'Toole and Raisbeck[4] have recently provided a comprehensive review of the various kinds of lesions associated with acute-subchronic and chronic selenosis in avian species. Other reviews of effects in adult birds also have been published recently.[8,13,19]

Laboratory studies have been conducted with mallards to determine the kinds of lesions and other measurements that can be used for diagnosis of selenium toxicosis in birds.[148,213,214] In general, ducks that received diets containing more than 20 mg Se/kg developed a number of lesions of the liver and integument. Those receiving 40 mg/kg or more selenium in their diets lost weight and had abnormal changes in the integument that involved structures containing hard keratin, such as feathers (alopecia/depterylation [i.e., feather loss]), beaks (necrosis), and nails (onychoptosis [soughed or broken]). When corroborated by elevated selenium concentrations in tissues (especially the liver), these integumentary and hepatic lesions, as well as weight loss, can serve for diagnosis of selenium toxicosis in birds. It should be noted, however, that some birds died without exhibiting any significant morphological lesions, even though they were emaciated.

Selenium toxicosis effects had been described previously in several species of aquatic birds found at Kesterson Reservoir from 1984 to 1986.[7,136,144] Those birds exhibited many of the same signs of selenosis as those later found in mallards (as described above) including hepatic lesions, alopecia, necrosis of the beak, and weight loss. As described in a previous section, most kinds of aquatic invertebrates at Kesterson had mean selenium concentrations greater than 100 mg/kg. Thus, the laboratory studies with mallards provided strong evidence that selenosis was the main factor responsible for morbidities and mortalities among birds at Kesterson Reservoir.[214]

In a 16-week exposure to evaluate susceptibility of mallards to over-winter mortality as a result of selenium exposure, ducks that received dietary concentrations of 20, 40, or 80 mg Se/kg (as selenomethionine) had higher mortality rates than ducks receiving the control or 10 mg Se/kg diets.[147] After 1 week of treatment, body weights were significantly depressed by the 20, 40, or 80 mg Se/kg diet. Ducks that survived for 16 weeks on the 20 mg/kg treatment were returned to a control diet; after 4 weeks, their body weight was similar to that of the control birds. Concentrations of selenium in blood were related to dietary treatment levels, but mortality was not clearly related to a threshold selenium concentration in the blood.

A number of studies (e.g., References 144, 215–218) have described physiological changes that are associated with selenium exposure in field-collected or laboratory-exposed birds. These generally involve changes in measurements associated with liver pathology and glutathione metabolism (e.g., glycogen, protein, total sulfhydryl and protein-bound sulfhydryl concentrations; glutathione peroxidase activity).

A few studies have suggested that effects of selenium on avian immune function are a sensitive endpoint and may affect birds at relatively low exposures.[219–221] However, immunotoxicity of selenium has not been studied sufficiently to provide a threshold-effect level in wild birds.[206]

17.4.2.5 Mammals

Most studies of the effects of excess selenium have focused on livestock and laboratory animals, and there have been no well-documented cases of significant selenosis problems among wild mammals.[17] The most common cause of chronic selenosis is consumption of forage containing more than 5 mg Se/kg. Sublethal effects were observed in dogs fed diets containing about 7 mg Se/kg.[2]

O'Toole and Raisbeck[4] have recently provided a comprehensive review of the various kinds of lesions associated with acute-subchronic and chronic selenosis in mammalian species, and they stress the importance of using multiple lines of evidence (e.g., selenium concentrations in tissues,

presence and types of lesions) in diagnosing selenosis. Subchronic and chronic selenosis (sometimes called "alkali disease") commonly involves changes in the integument, especially the hooves, horns, and hair. Loss of hair is a common sign of selenosis in a number of mammalian species and is similar to the bilateral loss of feathers observed in selenium-poisoned birds (described above).

Lesions of the liver occur in some species of mammals (sheep, dogs, cats, and rats), but they are not particularly significant in most livestock.[4] Unlike birds, congenital abnormalities are not commonly associated with selenium toxicosis in mammals. When congenital abnormalities occur, the female parent usually was exposed to near-lethal dietary concentrations of selenium.

At Kesterson Reservoir, which contained seleniferous agricultural drainage waters,[7] small mammals and raccoons were trapped and examined to determine whether selenium adversely affected the health of adult animals or their reproduction.[156,166] Despite very high concentrations of selenium in livers or whole-body small mammals, or in livers, hair, feces, or blood of raccoons, there was no evidence of adverse effects in the mammals. Parameters evaluated for the small mammals included body size, body condition, liver size, and reproduction (including examination for malformations). Those measures as well as histopathology of selected tissues (liver, kidney, and spleen) and blood chemistry were evaluated in the raccoons.

17.4.3 Interactions

Many studies have shown interactions between selenium and other inorganic elements (especially arsenic, sulfur, and various metals), vitamins A, C, and E, as well as sulfur-containing amino acids.[7,19,44,46,56,222] The interactions may be synergistic or antagonistic in terms of effects on uptake and metabolic effects, and the degree of interaction is affected by numerous factors. Thus, the topic of interactions is too complex to be addressed in detail in this review, and only a few examples of recent studies are discussed. Readers are encouraged to consult some of the references cited above for more details and a further introduction to this topic. Nevertheless, some of these interactions can be important factors in the design of field or laboratory studies and in the evaluation of results, and they should be taken into consideration.

Interactions between mercury and selenium have probably been studied more intensively in more kinds of animals than most of the others listed above. Mercury and selenium concentrations in the livers of various free-living carnivorous mammals often are highly correlated in a molar ratio of 1:1.[19,109,223,224] However, there is no consistent pattern for such a correlation in the livers of birds. For example, hepatic mercury and selenium were correlated, with an overall mercury:selenium molar ratio of 1:6, in diving ducks (surf scoters [*Melanitta perspicillata*] and greater scaups [*Aythya marila*]) from San Francisco Bay, California, during 1982.[140] In a subsequent study of scoters sampled twice at six locations in the Bay during 1985, mercury and selenium in the livers were not correlated; mercury:selenium ratios were typically between 1:7 and 1:15 for most locations and collection times, but the mean ratio at one site was 1:45.[225]

Elsewhere, mercury and selenium concentrations were positively correlated in some bird livers, but not in others, or they were negatively correlated (see review by Ohlendorf[226]). These relationships may change as birds remain at the sampling location (due to differential accumulation and loss rates for mercury and selenium), they may vary because of differing relative concentrations of the two elements, and other factors (such as the chemical forms present) also may complicate the patterns of bioaccumulation.

In the selenium-mercury studies discussed above, concentrations were reported for total selenium and total mercury. However, recent studies by Henny et al.[227] and Spalding et al.[228] have shown high correlations of selenium with inorganic mercury on a molar basis in fish-eating birds. Those authors suggested that selenium may contribute to the sequestration of inorganic mercury, thereby reducing its toxicity. This conclusion would be consistent with the results of a selenium-mercury interaction study with mallards by Heinz and Hoffman[208] discussed below.

Interactive effects of selenium with arsenic, boron, or mercury have been evaluated in reproductive studies with mallards.[124,125,208] Each of the studies involved varying levels of dietary exposures of breeding mallards to selenium alone, one of the other elements alone, and also selenium in combination with the other chemical. In each study, selenium and the other chemical caused significant adverse effects on reproduction when present alone in the diet at higher treatment levels, but the interactions varied by chemical. Antagonistic interactions between arsenic and selenium occurred whereby arsenic reduced selenium accumulation in duck livers and eggs and alleviated the effects of selenium on hatching success and embryo deformities. There was little evidence of interaction between boron and selenium when ducks were fed the two chemicals in combination. Selenium provided a protective effect that reduced the toxicity of mercury to adult male ducks. However, when the diet contained 10 mg Se/kg plus 10 mg Hg/kg, the effects on reproduction were worse than for either selenium or mercury alone. The number of young produced per female and the frequency of teratogenic effects were significantly affected by the combination of mercury and selenium in the diet, and mercury also enhanced the storage of selenium in duck tissues.

17.5 SUMMARY AND CONCLUSIONS

Selenium is a naturally occurring trace element that is essential for animal nutrition, but it becomes toxic to sensitive species of fish or birds at dietary concentrations about two or three times the background levels found in many food-chain organisms. The biogeochemistry of selenium is complex, and it is essential to understand selenium chemistry to enable competent predictions of its ecotoxicology. Selenium concentrations generally are elevated in areas where soils were derived from Cretaceous and Tertiary marine sedimentary rocks in the western United States. Irrigation of such soils has led to contamination of wetlands receiving drainage from the agricultural lands. Industrial sources (such as coal and oil production or combustion and phosphate mining) have contributed selenium to habitats where fish and wildlife can be exposed. Once released to the surface environment, selenium cycling is affected by many biogeochemical processes, especially in aquatic habitats.

Selenium bioaccumulates in aquatic and terrestrial food chains and in higher trophic-level animals that feed on those plants and animals. The greatest level of bioaccumulation is typically from water to aquatic plants or invertebrates (often a 1000-fold or greater increase occurs). Background concentrations have been fairly well established for plants and animals in freshwater and terrestrial ecosystems, and these values can be used in evaluating the selenium status of an area if field-collected samples are analyzed and compared to background values. Such studies have been conducted in a number of areas. Multitrophic-level laboratory studies also have been conducted and help interpret the significance of bioaccumulation of selenium.

Studies of the effects of selenium have focused mainly on domestic livestock and on fish and aquatic birds because those animals have been found to be sensitive to the adverse effects of excess selenium. In fish and birds, the early life stages (fish larvae/fry, bird embryos) are most sensitive, although lethal and sublethal adverse effects also occur in adult animals under field conditions in some areas. Teratogenic effects in fish larvae/fry and in bird embryos may lead to death of young that hatch, but a significant amount of mortality also may occur without visible developmental abnormalities.

Many studies have shown interactions between selenium and other inorganic elements (especially arsenic, sulfur, and various metals), vitamins A, C, and E, as well as sulfur-containing amino acids. These interactions may be synergistic or antagonistic in terms of effects on uptake and metabolic effects. The interactions of selenium and mercury often are of particular interest, and there is no universal rule of thumb as to the net result of these interactions. For example, in mallards, selenium provided a protective effect that reduced the toxicity of mercury in adult ducks, but the

effects of mercury plus selenium in the diet caused greater impairment of reproduction than either chemical alone.

Overall, there is enough information to thoroughly evaluate the significance of selenium as an environmental contaminant if it is included among the chemicals of potential concern when ecological risk assessments are planned and conducted.[204] The main difference between selenium and most other contaminants is that it is essential to have a good understanding of selenium's occurrence, complex biogeochemistry, and ecotoxicology to avoid serious errors.

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