Biogeochemistry and Analysis of Selenium and its Species

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Biogeochemistry and Analysis of Selenium and its Species

Executive Summary

The goal of this guide is to provide a background in analytical methods for environmental scientists responsible for assessing selenium (Se) concentrations in environmental (ores, soils, sediments, water) and biological samples. This guide summarizes Se chemistry, distribution, geochemistry, physiology and toxicology with the intention of directing attention to important considerations regarding chemical forms and their concentrations in environmental and biological samples that should be considered when performing Se analysis. Selenium chemistry results in generation of various molecular forms of Se in environmental materials such as soil and water, and Se's unique biochemistry produces a series of organic molecules with distinct physiological functions in plant and animal tissues.

Although Se is considered an environmental contaminant in locations where it occurs in excessive abundance, it is imperative to recognize Se's role as essential nutrient that is required to support life. All forms of animal life that have nervous systems require Se in their diet, to protect against oxidative damage and regulate redox balance in support of healthy brain, endocrine and immune functions. Although harmful effects accompany exposure to excessively high environmental Se, it is important to recognize that far more locations have adverse biological consequences because of too little, rather than too much Se present in the environment. Responsible management of environmental issues regarding Se requires environmental scientists to be aware of the full spectrum of concentration dependent effects of Se's complex biogeochemistry.

This guide focuses on appropriate analytical methods for performing reliable analyses of Se in environmental materials (ores, soils, sediments, water) and in various biological tissues. The relationship between environmental Se concentrations and the amounts of Se bioaccumulated from the environment are essential components of site-specific assessments of potential risks related to Se exposure. Risk assessment, management and remediation decisions need to be based on accurate and precise analytical data. The determination of Se and its species at ambient concentrations is complicated, and inappropriate analytical procedures have frequently been used. Analytical instrumentation and methods vary in their capability for assessing Se in various media, so it is important to recognize the relative strengths and weaknesses of different analytical methods. Selecting the best analytical approaches will make it possible to obtain the most reliable data regarding Se's concentration dependent effects in supporting normal physiology or potentially inducing toxicity. Analyses of total Se and Se speciation should be used in a complementary and comparative manner for risk assessment and remediation strategies.

Further detailed information regarding appropriate sample selection for evaluation of environmental toxicity of Se is presented in the Approach for Conducting Site-specific Assessments of Se Bioaccumulation in Aquatic Systems Workgroup effort being conducted concurrently with this guide.
Introduction

This manual provides essential background information to assist environmental managers and others interested in assessing concentration-dependent influences of Se's presence in environmental materials (ores, soils, sediments, water) on Se concentrations and molecular speciation in biological samples. The first sections of this guide discuss the biogeochemistry of Se in order to provide an understanding of Se chemistry, geological distribution, bioavailability, and biology, as well as the variety of molecular forms of Se in environmental and biological samples. The subsequent sections address instrumental analysis approaches and methods.

Instrumental approaches and recommended methods of performing Se analysis are described to familiarize environmental managers with the basis for the analytical methods. It is essential to recognize the specific advantages and limitations that accompany Se analysis using each of the various types of instrumentation. In these sections, there is a general discussion of the advantages and limitations of analytical approaches for specific sample types. The following sections describe sample specific considerations for each of the various material types encountered in environmental and biological Se assessments.

It is essential to understand the similarities and distinctions between the various molecular forms (species) of Se that occur in these sample types and how these forms are measured. Molecular forms of Se vary in stability in these various sample types and appropriate sample handling considerations for each sample types must be understood. In accordance with the official IUPAC definition (Templeton et al., 2000), the term “speciation” will be used in this document to describe the distribution of individual species within a sample, while the term “speciation analysis” refers to analytical methods and procedures employed to measure speciation.

Concentrations of Se referred to in this guide are in the standard international format of mg/kg. Please note that 1 mg Se/kg = 1μg Se/g = 1ppm Se. Although it is a convention (CGPM, 1971) to express all concentrations on a molar basis, we will refrain from this practice here, and express concentrations on a mass basis instead. If required (e.g., when comparing between different elements in an environmental or toxicological study), mass-based concentrations can be converted into molar concentrations by dividing by the atomic weight of Se (78.96 g/mol); i.e., 1g Se = 0.0127 mol Se. Selenium concentrations in tissues, geological raw materials, soils, and sediments are uniformly expressed in (mg Se/kg) on a dry weight basis in this report, and Se concentrations in water samples are expressed in (μg Se/L). When sample Se concentrations are described in this manual, it should be assumed they are on a total Se basis unless the concentration of a specific form is expressly being discussed. It is furthermore a convention that in speciation analyses, the concentration of each species is normalized to its Se content.
Historical Background

Selenium has a long history of being misunderstood and has repeatedly been mistakenly implicated as a toxicant, usually because of inadequate analytical data. Although today's analytical capabilities are much better, Se continues to be accorded less respect and appreciation than it deserves; even its name is usually mispronounced (the proper pronunciation is “si-LEE-ni-em”). Selenium was originally mistaken for tellurium (Te) until Jöns Jacob Berzelius recognized it was a distinct chemical element in 1818. Since it was obviously related to Te which was named after the Latin word for earth: tellus, Berzelius chose to name this new element selenium, in reference to selene, the Greek name for the moon goddess.

Selenium’s role in the environment first became widely recognized in the 1930s, but it was exclusively in association with toxicity (selenosis). A serious debilitating disease that afflicted horses and range cattle had been recognized since the early 1860s, and in the 1930s, it was suggested that this disease was the result of excessive Se exposure. This allegation and others that followed resulted in Se's name becoming generally associated with poisoning and toxicity. Even though many of these accusations have since been disproved, the numerous cases where Se was mistakenly found guilty by association have still given it a notorious reputation as an environmental toxin.

Two common livestock diseases known as "alkali disease" and "blind staggers" were initially blamed on Se toxicity. These diseases occurred in livestock that grazed in areas with Se rich soils. Certain types of range plants (popularly known as “loco weed” and “crazy weed” in the Dakotas & Wyoming) in the areas the animals grazed in contained remarkably high Se concentrations. Under the circumstances, it appeared that Se could have been the cause of the severe neurological damage and deaths that often were the result of these conditions. It had even been suggested that when Marco Polo described horses shedding their hooves and hair that he witnessed during his travels in China in 1295 that he might have been making the world's first report of Se toxicity.

However, Se does not appear to have been the proximate cause of toxicity in all of these cases. Until recently, selenosis was believed to be far more common than it actually is. For example, two-grooved milk-vetch (Astragalus bisulcatus), a native plant found on rangelands in western North America, does accumulate quantities of Se high enough to cause selenosis in cattle, horses, sheep, and swine (Baker et al., 1989). However, similar toxic effects (locoism) have been observed in animals that consumed related plants with far lower Se contents. These plants were found to contain swainsonine and other organic compounds that are extremely toxic (James et al. 1983). In controlled exposure experiments, sheep exhibited symptoms more appropriate to locoism (swainsonine poisoning) than to Se poisoning (Cheeke and Shull 1985). Although these other varieties of plants often had Se concentrations in the high range, it is now recognized that the blind staggers syndrome that afflicted these animals was not due to selenosis. While certain varieties of loco weed and crazy weed plants do accumulate notable amounts of Se, the pathological symptoms observed in livestock were often due to organic toxins rather than the Se they contained (O'Toole and Raisbeck 1995).
These plants contained alkaloids such as swainsonine and other neurotoxins, and the severe toxic consequences in the animals that ate them arose from exposure to these poisons. The suggestion that Se was the toxic agent responsible for the effects on livestock that Marco Polo observed appears to have been similarly mistaken. Many parts of China do have extremely high soil Se levels, but the hoof shedding and hair loss that Marco Polo observed in animals during his travels through Tibet and Western China occurred in regions that are not noted for high Se levels in plants. However the areas he traveled do have various plant types that contain alkaloids and other toxic organic substances that still regularly cause hair loss and hoof shedding in grazing animals (Shao and Zheng, 2008).

Similar mistaken assumptions that Se was causing animal toxicity have occurred in cases where Se was abundant in water, but not at toxic concentrations. This mistake appears to have been made because Se and sulfur (S) commonly occur together geologically. However, S concentrations often tend to be 100,000-1,000,000 times higher in environmental materials. According to the Merck Veterinary Manual, blind staggers occurs in animals that consume high-sulfate alkali water. Excess sulfate (>2% of diet) leads to the symptoms that had previously been attributed to selenosis.

In the absence of reliable Se analysis, unclear interpretations and mistaken assumptions have led to toxicological assessments that remain hard to understand. For instance, the term "alkali disease" appears to have originally described the toxic effects that occur after consumption of alkaline waters. These are now known to have been due to the toxic amounts of sulfate present in the water. However, the meaning of the term "alkali disease" has been shifted to retain its relationship with Se toxicity. The syndrome observed in livestock that consumed alkaline waters with toxic sulfate concentrations is now called blind staggers, and Se toxicity in livestock is now called alkali disease. Hair loss and shedding of hooves from selenosis are serious consequences in range animals, but there is often uncertainty regarding which cases are due to selenosis and which occur due to exposure to organic toxins in plants that also happen to be Se hyperaccumulators.

Even though Se did not cause many of the poisoning episodes attributed to it, its name still tends to be almost synonymous with poison in many people’s minds. Selenium's bad reputation is particularly unfortunate because most of its roles in the environment are beneficial rather than harmful, and there are far more locations where deficiency is a problem than locations where excess Se is an issue (Combs 2001).

This is not to say that selenosis does not occur as a result of high Se exposures. Selenosis from acute or chronically high Se exposures in food or water certainly causes debilitating symptoms and lethality. However, it is important to note that older literature contains misattributions of selenosis that have since been disproven. More recent analytical work and data interpretations are generally more trustworthy, and recent reviews (e.g., Ohlendorf et al, 2008) clarify understanding of concentration dependent effects of Se in the environment. Present and future studies of environmental Se that are based on more reliable analytical data will enable increasingly sophisticated interpretation.
Selenium Chemistry

Selenium, atomic number 34, is the third member of the Group 16 (old style designation VI.A) of the periodic table. It has an atomic mass of 78.96 and has six naturally occurring stable isotopes from 74-82. It shares fundamental aspects of its chemical behavior with oxygen (O₂) and S, the lighter members of the chalcogen family, as well as the heavier and less abundant Te, polonium (Po), and ununhexium (Uuh; synthetic element). Selenium's unique physical, chemical, and biological properties make it extremely interesting from a variety of perspectives. As seen in Figure 1, Se occurs in four redox states (VI, IV, 0, -II):

- Se(VI)  \( \text{SeO}_4^{2-} \)  selenate
- Se(IV)  \( \text{SeO}_3^{2-} \)  selenite
- Se(0)  \( \text{Se}^0 \)  elemental Se
- Se(-II)  \( \text{HSe}^- \)  selenide

Based on thermodynamic considerations, alterations in pH and redox conditions may cause shifts in equilibrium distributions between molecular forms designated by the compartments depicted in Figure 1, but whether or not these conversions actually occur in the environment depends on the kinetics (= speed) of their underlying reactions, and often ambient systems are not in thermodynamic equilibrium (i.e. one does not find the speciation predicted based on \( E_h \) and pH measurements alone). Organic Se species are also numerous and abundant in the environment. These molecular forms may also be sensitive to pH and redox changes in their milieu. Selenium's geologic distribution, chemistry and biochemistry all tend to be analogous to S.

**Figure 1.** Pourbaix diagram for selenium, predicting the thermodynamic stability of individual Se species as a function of system redox potential and pH (Drever, 1997).
Selenium Biogeochemistry

Selenium Geochemistry
Selenium is a constituent of 40 minerals and occurs as a minor component of 37 others, most being sulfides. The Se concentration in the Earth’s mantle is ~ 0.09mg Se/kg (NRC, 1983). Along with S, Se is vaporized at high temperatures so it is released in gaseous form whenever a volcano erupts. Upon cooling, Se condenses to form a layer upon ionized microparticulates that eventually precipitate in association with rainfall. High concentrations of atmospheric Se accumulate in bodies of water during active volcanic periods and are abundant in sedimentary rock laid down during Carboniferous, Triassic, Jurassic, Cretaceous and Tertiary Ages for this reason. North American Cretaceous shales commonly contain high levels of Se (Anderson et al. 1961). These shales are particularly rich in Se because an extensive inland seaway covered the central regions of North America from Mexico through Canada during the Cretaceous Age. This broad expanse of ocean was a down-wind repository of the Se and other materials released into the atmosphere during the period of great volcanic activity that characterized that age.

The sulfide ores of silver (Ag), copper (Cu), lead (Pb), mercury (Hg) and nickel (Ni) also tend to be rich in Se that can be released along with S during smelting of these metal ores. Uranium ores can contain as much as 600mg Se/kg. The heat of smelting is similar to volcanism in that Se and S are vaporized at these high temperatures and released in gaseous form whenever such ores are heated. As vaporized Se cools, it condenses onto ionized particulates that eventually fall to the ground or form a nidus for water vapor precipitation and fall with rain.

Selenium in Geological Raw Materials, Soils, and Sediments
Selenium generally occurs in relatively low amounts in geological raw materials (e.g. rocks, ores, coals, crude oils), soils, and sediments (ranking 69th in elemental abundance). Selenium concentrations in rocks are generally comparable to those found in soils and sediments (low mg/kg), but Se concentrations in coals and crude oils can reach hundreds of mg/kg in certain cases (depending on the geological evolution of the deposit). Selenium concentrations in ores can be > 50% (= 500,000 mg/kg) for selenide minerals, and are often in the low % (= 10,000 mg/kg) range for sulfide minerals; in other types of ores, Se concentrations are generally much lower. Selenium concentrations in soils and sediments vary geographically depending on the parent rock. Concentrations of Se in soil can vary dramatically over short distances depending on differences in the bedrock from which they are derived and influences of leaching effects. Hilltop soil Se levels can be much lower than those seen in adjacent low lying areas. Soil Se varies from 0.01mg/kg in deficient areas to 1,200mg/kg in organic rich soils in toxic areas (Keller, 2000).

Because soils of the central region of North America are formed from Cretaceous shales, this region tends to be rich in Se (see Figures 2 and 3A). The central region of North America not only has rich amounts of Se in its soils, its soils also have favorable pH characteristics that increase Se availability for uptake. Figure 3B shows that certain parts of North America that have reasonably rich soil Se levels will still have poor Se uptake in plants that form fodders
for livestock in those regions. This is often the result of low pH conditions that make inorganic Se less mobile and available for uptake. These effects are most apparent in the coastal and mountainous areas of North America. Centralized food distribution networks tend to moderate these regional effects so the Se status of people in low Se areas is not necessarily affected. Even so, blood Se concentrations across North America show a generalized plateau of rich Se status in the midsection of the continent and lower levels on both of the coasts.

Se (ppm) in <2 mm fraction of Surface Soils

Figure 2. Selenium distribution in soils of South-Central Canada (Garrett, 1997).

Countries of Northern and South Central Europe generally tend to have much less Se present in their foods than is common in North America. Finland and New Zealand were formerly recognized as having very low Se levels. However, when this was recognized, Finland began adding Se to their fertilizers (Na2SeO4 added at 16g Se/ton to cereal fertilizer and 6g Se/ton of forage crop fertilizer) in 1985, later changed to 6g Se/ton for all fertilizers. The Se content of spring wheat increased 15-20 fold and Se in milk, meat, and eggs increased 3 to 9 fold by 1986 (Aro et al., 1988). The blood Se levels of their population gradually increased from ~80µg/L in 1985 to ~130µg/L by 1989 and held steady at that level (Mäkelä et al., 1993). New Zealand also augmented the Se in their soils and animal feeds and rapidly enhanced their Se status (Reilly, 1996). Much of Africa has regions with extremely low Se, and Asia, particularly China, is characterized by having both highly exposed and severely Se deficient populations. More regions of the world are characterized by moderate to low Se bioavailability than rich or high soil levels (Combs, 2001).
Figure 3. Selenium distribution and availability in regions of the continental United States. Panel A (from Gustavsson et al., 2000) indicates the soil Se concentrations in regions of the continental United States. Panel B (from Rosenfeld and Beath, 1964) shows plant Se concentrations in the same regions.
For the purpose of analysis, a soil is defined as abiotic (non-living, but potentially containing biotic materials in various stages of decomposition) solid matter that is not covered with water, while a sediment is defined as abiotic solid matter that is temporarily (e.g. a wetland) or permanently covered with water. Ignoring local Se emission scenarios, there is no reason to assume any fundamental difference between Se concentrations in soils vs. sediments, provided one takes into account that sediments are generally saturated with water; consequently, all Se concentrations in solids should be expressed on a dry weight basis to eliminate these differences. Generally, aside from establishing local background concentrations, the interest in studying Se in soils and sediments is limited to materials that show (or have the potential to show) elevated Se concentrations as the result of anthropogenic activities. Se concentrations in sediments would be elevated above the local geogenic background (which may be high in certain geological settings) as the result of Se deposition from the overlying water body, while soils would have elevated Se concentrations as the result of atmospheric deposition and/or leaching of Se from residual industrial materials deposited on top of them.

In all of these assessments, it is often crucial to define if a solid material is “contaminated”, i.e. if its Se concentration is above the local background. However, it is impossible to know what an appropriate local background Se concentration is without studying it explicitly. Geoscientists often refer back to average crustal background values for trace elements, usually citing a reference publication by Turekian and Wedepohl (1961), which lists a Se background concentration of 0.05 – 0.08mg/kg in igneous and sedimentary rocks, but up to 0.6mg/kg in certain shales. It has to be emphasized, though, that this is a global background averaging all types of geological materials, and has no practical meaning for the assessment of any specific locality.

In practice, local Se backgrounds depend strongly on the composition and geological history of the particular soils or sediments under investigation, and may vary by several orders of magnitude. The other aspect of evaluating the Se concentration of a soil or sediment is putting it into the context of potential ecotoxicological effects that might arise if organisms are exposed to them. If the Se concentration exceeds the level where the occurrence of ecotoxicological effects is expected, then these materials are referred to as “polluted”. In practice, this is generally done by comparison to local regulatory guidelines, which in turn depend strongly on the intended use of the soil/sediment and on the potential exposure that organisms may have to these materials. For example, the Canadian Soil Quality Guideline for Se is 1.0 mg/kg for agricultural, residential and park lands, and 3.9mg/kg for industrial and commercial lands. By comparison, the “natural” Se concentration of Canadian soils was found to range from 0.03 to 2mg/kg, with an average of 0.26mg/kg (EC, 2002). In fact, most North American soils are Se deficient, so that Se pollution of soils is not a common issue, except in cases of direct anthropogenic emissions to the soils. Likewise, the Se sediment quality guideline in British Columbia is 2mg/kg (Nagpal and Howell, 2001), which is thought appropriate for prevention of Se bioaccumulation in aquatic food chains. The natural Se background of freshwater sediments is assumed to be 0.29mg/kg (Buchman, 2006), and is thus comparable to the assumed soil background.
Se in soils or sediments is thought to exist in several different forms. Contrary to waters, it is not always possible to assign an exact chemical structure to these binding forms, so the term “speciation” should be used with some care to describe Se binding forms in these samples. The term “fractions” may be more appropriate, because different individual chemical forms may show the same spectroscopic or extraction behavior (see section on Se speciation analysis in these matrices below). Nonetheless, Se is found in the forms of selenite and selenate dissolved in the pore waters and adsorbed to minerals surfaces (particularly iron (Fe) and manganese (Mn) minerals) of soils and sediments. Under reducing conditions, other discrete inorganic Se ions may also exist in these compartments. Under more reducing conditions, especially in deeper sediments, Se is believed to mimic the chemistry of its homolog S, so elemental Se⁰ is postulated to be a major Se species. Finally, there is some evidence that Se may be associated with sulfide minerals in the form of selenide (Se⁻²). As Se is generally present in low concentrations, it is not likely that it will form any discrete Se minerals in soils or sediments, but will rather be found associated with major element minerals.

Organic Se compounds exist in soils and sediments, but their exact chemical nature is unknown. Contrary to biological tissues (see below), there is no analytical evidence that supports the existence of free low-MW organo-Se compounds as significant Se fractions in soils or sediments. Likewise, the classic concept of trace elements being complexed by natural organic matter (NOM) in soils and sediments (developed for cations like Pb and Cu) is not applicable to Se, because most inorganic Se compounds are anionic at ambient pH, and are thus thought to be electrostatically repelled by NOM. Since Se is an essential trace element found in most organisms, it is hence more appropriate to assume that “organic Se” in soils and sediments is covalently bound in the decomposing NOM as a remnant of its original speciation in the living tissue.

Selenium in Waters
Typical Se concentrations in ambient waters are < 1µg/L in the absence of direct Se sources. The background Se concentration of marine waters is 0.02 – 0.04µg/L; it has not been determined systematically what a corresponding background concentration would be for fresh waters, but is seems reasonable to assume that it would be comparable to the marine reference value. In waters under the impact of geogenic or anthropogenic Se emissions, Se concentrations are typically in the range of 1-10µg/L, and can occasionally exceed 100µg/L in exceptional cases. Se in industrial effluents can exceed 1,000µg/L under rare circumstances, but is usually in the 10 – 100µg/L range.

Obviously, Se concentrations in industrial effluents depend strongly on the applied treatment technologies and the overall water management at the facility. Likewise, Se concentrations in the receiving environment depend strongly on the mixing ratio of discharge stream to receiving water, as well as the overall fate of Se after discharge, particularly the relative importance of Se removal to the sediments compared to its dispersion in the aqueous phase.
Selenium water quality criteria are primarily driven by ecotoxicological considerations for organisms at the top of aquatic food chains (e.g., waterfowl and predatory fish); current regulatory guidelines include 5µg/L in the US and 1µg/L in Canada (with varying guidelines up to 100µg/L in individual provinces), but these are usually not enforced, due to a lack of laboratories who can reliably measure Se at these concentrations, as well as considerations related to availability and economic feasibility of suitable treatment options. Additionally, due to the complexity of the environmental biogeochemical Se cycle, there is usually not a straightforward relationship between Se concentrations in waters and the observed ecotoxicological effects (or lack thereof), so new initiatives are underway to base regulations on sediment or tissue Se concentrations instead.

In oxic surface waters, Se typically exists in the form of two major species: the oxyanions selenite (HSeO$_4^-$; oxidation state +IV) and selenate (SeO$_4^{2-}$; oxidation state +VI). Thermodynamically, selenite is instable and would be predicted to convert into selenate over time, but in practice, the kinetics of this reaction are so slow that it does not occur in ambient waters, unless microbes are involved. Nonetheless, well-oxidized waters tend to contain mostly selenate, and as the redox potential decreases (e.g., towards the sediment-water interface and into the sediment pore waters), selenite initially increases in terms of relative importance over selenate. However, contrary to selenate, selenite adsorsbs strongly to the surface of Fe and Mn hydroxide minerals, so it may be lost from the aqueous phase, leaving a Se pattern which could be uncharacteristic of the system’s redox state. Additionally, selenite (as well as selenate) can be further reduced to insoluble elemental Se (especially by microbial activity), so in strongly reducing waters, neither selenite nor selenate may be encountered.

Some unpublished evidence also suggests that other soluble inorganic Se species besides selenite and selenate may be formed in strongly reducing waters by reaction of Se species with reduced S compounds. Other Se species (specifically various discrete or operationally-defined organic Se compounds) have been determined or postulated to exist in ambient waters, but they generally constitute only minor fractions of the total Se concentration in oxic surface waters. This makes them negligible for the overall Se cycling and fate, but may potentially have implications for uptake by and ecotoxicity to aquatic organisms (although this is not proven yet). Selenium is not very particle-reactive, so particulate Se is not very important from a quantitative point of view in oxic waters.

The current acute US EPA Se criterion for fresh waters is actually based on Se speciation information (USEPA, 2008). It recognizes the different bioavailability of selenite and selenate, and calculates a site-specific acute criterion as $f[\text{Se(IV)}] \times 12.83\mu g/L + f[\text{Se(VI)}] \times 185.9\mu g/L$, where $f$ means the relative fraction of each species. This criterion has to our knowledge never been implemented or enforced for an impacted water body, but there are some regulatory bodies that have asked affected stakeholders to include Se speciation information in the monitoring efforts for their effluents. Finally, there are several efforts underway to establish site-specific water quality objectives for a number of different ecosystems subjected to Se emissions, and Se speciation is considered as one key factor in the establishment of such guidelines.
Selenium in Plants
Selenium enters the food web when plants accumulate it from soil and incorporate it during synthesis of new molecules, typically as Se-substituted analogues of thiomolecules. Selenomethionine is the major organic selenomolecule in cereal grains, legumes and soybeans, as well as in Se-enriched yeast used in Se supplements, but a number of other organic and inorganic molecular species are also present.

Although plants have no known physiological requirement for Se, many plant species accumulate large amounts of it from soil and water. Plants can be divided into three groups based on their capacity for accumulating and tolerating Se (Rosenfeld and Beath, 1964; Terry and Zayed, 1998). Primary accumulators (hyperaccumulators), such as some *Astragalus*, *Stanleya*, and *Xylorhiza* species, are able to accumulate several thousand mg Se/kg of leaf tissue. These species preferentially grow on seleniferous soils and often contain levels of Se that are toxic to horses and cattle. Secondary accumulators (Se absorbers), such as some *Brassica* species, can accumulate up to 1,000mg Se/kg of leaf tissue. These species are not confined to seleniferous soils, but are able to accumulate Se if it is present. Nonaccumulators usually do not accumulate more than 50mg Se/kg under field conditions. This group includes most grains and grasses.

Selenium is taken up by plants as selenate, selenite, and organic Se (Terry and Zayed, 1998). The presence of sulfate is a limiting factor which influences the uptake of Se by most plants. Additionally, it has been suggested that phosphate concentration influences selenite uptake (Hopper and Parker, 1999), which may hint at a common uptake pathway. It is believed that plants other than hyperaccumulators take in Se in place of sulfate. Selenium entering a plant as selenite can be reduced and converted to selenocysteine. When selenoamino acids such as selenocysteine are incorporated into proteins, protein dysfunction can result, which may be one mechanism for toxicity (Brown and Shrift, 1982). Hyperaccumulators take in Se regardless of the presence of sulfate (Terry and Zayed, 1998). It is believed that they possess an alternate pathway for uptake and retention of Se. Selenium tolerant plants produce large amounts of nonprotein amino acids (Se-methylselenocysteine and selenocystathionine). It is possible that high rates of synthesis of these amino acids may be a means of Se detoxification.

There is some evidence that Se may have a role in countering UV-B stress in plants. Addition of Se to low Se soil has been shown to alleviate UV-induced oxidation damage under greenhouse conditions (Hartikainen and Xue, 1999), to improve the recovery of chlorophyll from light stress (Seppanen et al., 2003), and to increase the antioxidative capacity of senescing plants (Xue et al., 2001). In field studies of the effects of Se addition on UV-B stress of strawberry plants, Se did not ameliorate the harmful effects of UV-B, but increased leaf growth was observed with a low level of added Se (Heijari, 2006).

Selenium hyperaccumulation may protect plants from herbivory and fungal infection. Selenium has been shown to protect Indian mustard (*Brassica juncea*) plants from herbivory by caterpillars (*Pieris rapae*) (Hanson et al., 2003). Selenium containing plants were also found to be less susceptible to a root/stem pathogen (*Fusarium* sp.) and a leaf pathogen (*Alternaria brassicola*). However, in the same study, snails (*Mesodon ferrissi*) showed no signs of toxicity at the same Se levels toxic to caterpillars and actually seemed to prefer leaves.
containing Se. Selenium protects plants from insect feeding both by deterrence and toxicity (Hanson et al., 2004). Green peach aphids (*Myzus persicae*) are able to detect and avoid Se-containing leaves with levels as low as 10mg Se/kg dry weight. In non-choice feeding experiments, aphid population growth was inversely correlated with leaf Se concentration.

Some insects have evolved resistance against the Se hyperaccumulation defense of plants. A newly discovered variety of the diamondback moth (*Plutella xylostella*) thrives on prince’s plume (*Stanleya pinnata*) plants containing levels of Se which are highly toxic to susceptible varieties of the same insect species (Freeman et al., 2006). A Se-tolerant wasp (*Diadegma insulare*) parasitizes the tolerant moth. Both the moth and its parasite accumulate methylselenocysteine, the same form found in hyperaccumulator plants, while related susceptible moths accumulate selenocysteine.

The Se contents of plants are highly dependent upon the Se content of the soil they grow in. For this reason, plants grown in the Great Plains of the USA and Canada are rich sources of Se, whereas plants grown in the soils of the northeast, northwest and Florida are lower (see Figure 3 B). The Se-content of soils in Europe and the Se blood levels of Europeans are generally much lower than what is seen in North America. Plant Se concentrations are particularly low in plants grown on native soils of Finland, New Zealand and central parts of China. Leaching of Se into freshwater sources from soils treated with agricultural fertilizers containing Se was studied by Wang et al. (1995). They found lakes surrounded by fertilized fields had higher Se contents than forest lakes in late summer, but not in spring.

**Selenium in Animals**

Selenium is toxic to animal life when it occurs in sufficiently high concentrations. Even though its geological concentrations are normally quite low, its toxic threshold is also lower than for most other elements commonly considered as toxins in the environment (Keller, 2000). When selenomethionine and other organo-Se compounds are consumed by animals, they tend to be well absorbed and non-specifically incorporated into proteins in place of methionine, but selenomethionine serves as a reserve supply of Se for selenocysteine. Selenite and selenate (preferred inorganic form) are also readily absorbed and rapidly converted into selenocysteine in vivo (Whanger, 2002). All these selenomolecules are eventually catabolized into hydrogen selenide and either used in de novo synthesis of selenocysteine or methylated and exhaled in breath as dimethylselenide or excreted in urine as the trimethylselenonium ion (Janghorbani et al., 1999). Two of the amino acids that occur in proteins are distinguished by possession of Se: selenomethionine and selenocysteine. Selenomethionine is biochemically equivalent to methionine and is chiefly regarded as an unregulated storage compartment for Se. In contrast, selenocysteine synthesis is genetically regulated and it is specifically incorporated into numerous proteins that perform essential biological functions. Dietary Se occurs in a variety of molecular forms, but the biochemical distinctions between the amino acids selenomethionine (predominant form present in plants) and selenocysteine (actively synthesized in animal tissues) are particularly significant.
Figure 4. General scheme of vertebrate Se metabolism (Ralston et al., 2008).

Protein synthesis cycles make no distinctions between selenomethionine and methionine (see Figures 4 and 5), but upon eventual degradation, the Se freed from selenomethionine becomes available for synthesis of selenocysteine in animal cells. In contrast to other amino acids, selenocysteine is normally not reused in subsequent cycles of protein synthesis. Instead, before a new selenoprotein can be created, a pre-existing selenocysteine molecule must be degraded to release its Se, and/or synthesis of a completely new selenocysteine must be performed. However, there is evidence that selenocysteine can be reincorporated into proteins in place of cysteine (Unrine et al., 2007; Moroder, 2005).

In the nutritionally relevant range, dietary selenium that is readily bioavailable for selenocysteine synthesis counteracts the neurotoxicity of Hg, cadmium (Cd), Pb, and vanadium (Whanger, 2001) and Se status is inversely related to arsenic (As) induced cancers (Chen et al., 2007). These elements have higher binding affinities for Se than for S, e.g. a million times higher affinity between Se and Hg than between S and Hg (Dyrssen and Wedborg, 1991), and mass action effects appear to drive formation of metal selenides. In the case of Hg, the mechanism of toxicity appears to occur through irreversible inhibition of selenoenzymes as a result of direct formation of complexes with selenocysteine at the active sites of the enzymes (Ralston et al., 2008). This same mechanism may also occur in other metal toxicities and could be a contributing factor in As carcinogenicity.
Figure 5. Structures of sulfur- and selenium-amino acids.

Although selenocysteine is structurally analogous to cysteine, it is genetically, biochemically and functionally unique. Cysteine’s thiol is protonated (pKₐ ~ 8.5) at cellular pH and the reduction potential of cysteine’s S is not as great as that of selenocysteine’s Se. The catalytic activities of Se-dependent enzymes (selenoenzymes) depend upon the biochemistry of the selenocysteine present at their active sites (Behne et al., 2000). The unique capabilities of the various selenoenzymes (see Table 1) occur because selenocysteine’s highly negative redox potential enable it to conduct reactions that cysteine cannot accomplish. Furthermore, because selenocysteine's selenol is ionized (pKₐ 5.7) at physiological pH, it is also more biochemically active.

Table 1. Mammalian selenoproteins

<table>
<thead>
<tr>
<th>Selenoprotein</th>
<th>Forms and functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidases</td>
<td>4 forms; detoxification of peroxides in cytosol and lipid compartments</td>
</tr>
<tr>
<td>Thioredoxin reductases</td>
<td>3 forms; regenerates thioredoxin, Vitamin C, and Vitamin E</td>
</tr>
<tr>
<td>5′ Deiodinases</td>
<td>3 forms; converts T₄ into T₃ (thyroxine), regulates thyroid hormone status</td>
</tr>
<tr>
<td>Selenophosphate synthetase</td>
<td>Catalyzes formation of selenophosphate for selenocysteine formation</td>
</tr>
<tr>
<td>Methionine R-sulfoxide reductase</td>
<td>Reverses oxidative damage to R-methionine (formerly known as SelR and SelX)</td>
</tr>
<tr>
<td>Selenoprotein P</td>
<td>Transports Se to the brain (10 selenocysteines per molecule) and other tissues</td>
</tr>
<tr>
<td>Selenoprotein S</td>
<td>Influences inflammatory response</td>
</tr>
<tr>
<td>Selenoprotein N</td>
<td>Unknown function, but deficiencies associated with muscular dystrophy type 1</td>
</tr>
<tr>
<td>Selenoprotein W</td>
<td>Appear to regulate redox state of 14-3-3 proteins (1% of total brain protein)</td>
</tr>
<tr>
<td>Selenoprotein T</td>
<td>Appear to regulate redox state of 14-3-3 proteins</td>
</tr>
<tr>
<td>Selenoprotein V</td>
<td>Appear to regulate redox state of 14-3-3 proteins</td>
</tr>
<tr>
<td>Selenoprotein H</td>
<td>Oxidoreductase that regulates 14-3-3 proteins in nucleoli</td>
</tr>
<tr>
<td>Selenoprotein M</td>
<td>Oxidoreductase that assists in protein folding in endoplasmic reticulum</td>
</tr>
<tr>
<td>Selenoprotein 15</td>
<td>Oxidoreductase that assists in protein folding in endoplasmic reticulum</td>
</tr>
<tr>
<td>LMW selenomolecules</td>
<td>Functionally uncharacterized, but abundant in normal brain tissues</td>
</tr>
</tbody>
</table>

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a Information presented in this table compiled from Gladyshev et al., 2004; Moghadaszadeh and Beggs, 2006; Dikiy et al., 2007; Aachmann, et al., 2007; Linster and Van Schaftingen, 2007.
Selenoenzymes use the extensive redox potential of selenocysteine strategically positioned in their active sites to perform their functions including apparently indispensable antioxidant functions in the brain (Chen and Berry, 2003; Schweizer et al., 2004). These functions may explain why virtually all forms of animal life that possess nervous systems express and preserve selenoenzyme activities in their brain and neuroendocrine tissues (Behne et al., 2000). Selenium and selenoenzyme levels in these tissues are maintained at near normal levels, even when somatic tissue Se levels become virtually depleted.

Selenium deficiencies in animal herds have occurred throughout the United States and have been recognized in many other regions of the world. In the United States, Se deficient areas have been found in 44 states. Because humans tend to consume foods that originate from broader areas, Se deficiencies are relatively rare among people. However, in cultures where locally grown foods are dominant in the diet, Se deficiencies can occur. In regions with low Se, compromised immunity appears to result in increased incidence of viral diseases such as HIV and Hepatitis C, and contributes to mutation of these viruses into new forms.

The standard of recommended intake levels of Se is under debate (for a full review, see Rayman, 2000). The UK reference nutrient intake (RNI) of 75μg per day for men and 60μg per day for women has been determined as the amount believed to be necessary to maximize the activity of the antioxidant glutathione peroxidase (GPx) in plasma (MacPherson, 1997). The American recommended dietary allowance (RDA), set at 55μg per day for both men and women, is based on the investigations of the Se intake required to achieve plateau concentrations of plasma GPx (Standing Committee, 2000).

The WHO/FAO/IAEA expert group recommended an intake level of only 40μg per day for men and 30 μg per day for women, assuming only two-thirds of the full expression of GPx activity is required (WHO, 1996). However, as Rayman (2000) points out, if levels of GPx activity saturation are determined using platelets rather than plasma, then the intake levels needed should be approximately 80-100μg per day.

Additionally, intake levels which saturate plasma GPx activity are insufficient to optimize the immune response and reduce cancer risk. This insufficiency is amplified at intake levels suggested by the WHO/FAO/IAEA which only support two thirds of plasma GPx activity. Currently, the UK and other European countries have intake levels of approximately half the RNI, and Se-poor regions of China have intakes of less than 19μg per day for men and less than 13μg per day for women.

Selenium acts as a growth factor; has powerful antioxidant and anticancer properties; and supports normal thyroid hormone homeostasis, immunity, and fertility. Selenium deficiency arises because of compromised selenoprotein production, resulting in diminishment of all these functions. However, Se toxicity does not appear to occur because of excessive amounts or activities of these enzymes.
Selenium Toxicity
Selenium homeostasis is the process by which the body controls internal Se balance by retaining more when the supply is short, and excreting more when there is too much in the body. However, it is not always possible for the body to excrete as much as is coming in. When this happens, Se toxicity will occur. Since the physiological basis of selenosis has not been defined and biochemical indicators have not been identified, it is difficult to distinguish levels of Se that are safe and beneficial from those that are potentially harmful to health. Selenosis in mammals is characterized by brittle hair and nails, skin lesions, and mental dullness. However, these symptoms disappear when Se intake is reduced because the body can excrete the excess and restore homeostatic balance.

Selenium toxicity is rare in humans, although cases have been reported. Selenium poisoning was reported in 13 persons in the United States who consumed a supplement that contained 182 times more Se than stated on the label (Helzlsouer et al., 1985). The total amount of Se ingested by the victims was calculated to be 27–2,387mg. The most common symptoms were nausea, vomiting, hair loss, nail changes, irritability, fatigue, and peripheral neuropathy. Chronically intoxicated individuals ingested an average of 4.99mg Se/day, with some individuals consuming as much as 38mg Se/day in Enshi County, China (Yang et al., 1983). Signs of selenosis included loss of hair and nails, skin lesions, tooth decay, and abnormalities of the nervous system.

For each animal species there is a narrow range of Se concentrations that are high enough to be adequate, but low enough to not be toxic when chronically consumed. Among mammals these ranges tend to vary from 0.1-2mg Se/kg, but the range of transition from nutritional adequacy to toxicity is dependent on dietary form. This results in a factor of 20 between concentrations that meet dietary requirement and those that will cause initial onset of toxicity. In animal studies, chronic consumption of 4-5mg Se/kg diets causes growth inhibition and result in tissue damage (Hafeman et al., 1974). The acute toxicities of various forms of Se have been studied in animals using graduated injections (mg/kg body weight) to establish the lethal dose that results in 50-75% lethality.

Dimethylselenide is an uncharged and volatile molecule, so it is readily excreted in breath. This is one of the natural ways by which excess Se is exported from the body. When large amounts of Se are consumed, more dimethylselenide is exhaled, resulting in a perceptible garlic odor in the breath. The high levels of dimethylselenide that are required to induce toxicity may occur because it employs an existing excretion system for elimination. The lethal toxicity of the other Se species is far greater, perhaps because processes that can metabolize these species for elimination require an induction period to develop efficient biochemical means for chemical modification and excretion. It is known that animals that are chronically exposed to moderate amounts of toxins are less vulnerable to toxicity of acute doses, but this adaptive effect has not been adequately studied in relation to Se toxicity.

It is important to note that all of these acute toxicities cited in Table 2 occur at substantial excess over normal biological Se concentrations. For comparison, the U.S. recommended daily (RDA) amount of Se (70μg Se/day) divided by the average weight of American males (79kg) results in 0.9μg Se/kg body weight shown in Table 2. The No Observed Adverse
Effect Level (NOAEL) established from human populations in China (853μg Se/day) divided by the average body weight of the Chinese (55 kg) results in the 15.5μg Se/kg body weight also shown in Table 2. Therefore, in humans there is a ~20 fold factor between the dietary intakes considered adequate and the threshold of exposures that result in toxicity. This factor is virtually identical to what has been observed in other mammalian models. It is not known whether this factor is the same in other vertebrate species, and aside from a few instances, e.g. Brix et al., (2001) invertebrates remain poorly studied. This is unfortunate since invertebrates form the base of both aquatic and terrestrial food chains.

Table 2. Relative toxicities of different selenium species

<table>
<thead>
<tr>
<th>Selenium species</th>
<th>mg Se/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD50/75 in rats</td>
<td></td>
</tr>
<tr>
<td>sodium selenite</td>
<td>3.25-3.5</td>
</tr>
<tr>
<td>sodium selenate</td>
<td>5.5-5.8</td>
</tr>
<tr>
<td>D,L-selenocysteine</td>
<td>4</td>
</tr>
<tr>
<td>D,L-selenomethionine</td>
<td>4.3</td>
</tr>
<tr>
<td>diseleno-dipropionic acid</td>
<td>25-30</td>
</tr>
<tr>
<td>trimethylselenonium</td>
<td>49</td>
</tr>
<tr>
<td>dimethylselenide</td>
<td>1,600</td>
</tr>
</tbody>
</table>

Nutritionally relevant range in humans

- Human Se intake: U.S. RDA 0.0009 (optimal intake level)
- Human Se intake: NOAEL 0.0155 (high, but harmless intake level)

The Kesterson Reservoir was a well studied example of Se toxicity in the environment (Ohlendorf and Santolo, 1994). This artificially developed wetland designed to collect water from subsurface tile drainage from irrigated agricultural lands of the San Joaquin Valley was completed in 1978. High Se soils in this area can be greater than 2mg Se/kg, and effects of leaching from these soils resulted in heavily contaminated waters accumulating in the reservoir. Fish began dying in the reservoir in 1981, and in 1983 field observations showed dead and deformed embryos and hatchlings of several species of aquatic-dependent birds. Selenium was identified as the probable cause of the deformities in the embryos and chicks. Drainage water entering the reservoir contained an average of 0.35mg Se/L and occasionally as much as 1.35mg Se/kg. Since Se toxicity has been noted in the microscopic organisms that comprise the base of the aquatic food chain with concentrations as low as 0.01mg Se/L, the waters of the Kesterson Reservoir were outside the range associated with ecological health. For the most recent evaluation of current information and considerations for proper risk assessment of environmental Se see the review by Ohlendorf et al. (2008).

However, it is difficult to establish a reliable water safety criterion without having a defined mechanism of toxicity that can readily be assessed in association with biological indicators. Presently, the postulated mechanisms relate to Se interactions with thiols of critical proteins, but none of the current studies have identified discrete molecular mechanisms that could be used to define the pathology of Se toxicity. Accumulation and incorporation of selenocysteine in proteins (Unrine et al., 2007; Moroder, 2005), and perhaps more
particularly, into intermediate substrates such as glutathione and thioredoxin, could disrupt physiological pathways because of differences in reaction potentials between thiols and selenols (Raisbeck, 2000). Selenocysteine incorporation into proteins could compromise disulfide bridges within/between proteins and peptides. Therefore, this mechanism has potential to explain hair and hoof lesions of selenosis. Alternatively, inorganic Se may directly interact with thiols to spawn free radicals (Hoffman, 2002; Kaur et al., 2003; Balogh et al., 2004) that damage cells.

**Instrumental Methods for Analysis of Selenium and its Species**

Analytical techniques commonly employed for the determination of Se in environmental samples include inductively-coupled plasma-mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS) and atomic absorption spectrometry (AAS). The latter two are generally used in combination with hydride generation (HG) as a sample introduction method, and AAS is additionally used with graphite furnace sample atomization (GF-AAS) for specialized applications. Other analytical techniques that are used occasionally, but are not widespread in their application, include instrumental neutron activation analysis (INAA), inductively-coupled plasma-optical emission spectrometry (ICP-OES), electrochemical methods and fluorometry (Wang et al., 1994).

**General Quality Control Requirements**

It is important to understand that officially-approved methods and/or a general laboratory accreditation do not guarantee high data quality for total Se and Se speciation measurements, so the data recipient/user must find other ways of convincing themselves of the suitability of specific laboratories or analytical methods for these tasks, e.g. by seeking expert advice. Accordingly, all analytical and sample treatment procedures need to be described in sufficient detail; if officially-approved or previously published methods are employed, any deviation from the procedure should be documented. In addition to the more specific QC standards described for every major analytical technique and sample matrix below, the stakeholder should expect the analytical laboratory to provide at the minimum the following information with all analytical results to allow the independent evaluation of data quality. Stakeholders should avoid laboratories that cannot meet these requirements.

For the analytical measurement, the laboratory should analyze at least three blanks and estimate a method detection limit (MDL) based on those. Selected samples should be analyzed in replicate to estimate analytical reproducibility, and should be spiked in replicate to assess instrumental accuracy and reproducibility of the spiking procedure. Certified Reference Materials (CRMs) suitable for (i.e. matching closely) the particular samples (as far as possible) should be analyzed to check accuracy. Recurring sensitivity checks (by analyzing standards) should be performed during analytical runs (specifically longer ones) to detect any instrumental drift, and if this drift is $\geq 10\%$ throughout a group of samples, it should be compensated for (and the employed compensation procedure documented).
Finally, calibration curves should be validated by comparison of the concentration determined using the curve for an independent standard (different lot number) to its known concentration. The observed concentration for this standard should not deviate more than 10% from the theoretical concentration.

For any type of sample that requires pretreatment prior to analysis, e.g. digestion or extraction of solid samples or derivatization of liquid samples, the laboratory needs to analyze separate blanks, blank spikes and sample spikes to assess the quantitativeness of these steps, and thereby the accuracy of the overall method. Proper MDLs (including all dilutions and corrections) need to be provided. Selected samples should be processed in replicate to obtain an estimate of sample inhomogeneity (this variability will generally be significantly larger than the analytical reproducibility for all types of solid samples!). All CRM and spike results should be reported in the form of a recovery relative to the certified/known value. All analytical results should be rounded sensibly, with the number of significant figures appropriate for the reported detection limits and reproducibility, but not to exceed three!

**ICP-MS for Analysis of Selenium**

The advantages of inductively coupled plasma-mass spectrometry (ICP-MS) for analysis of Se include sensitivity and speed; however, great care must be taken to avoid systematic bias. An ICP-MS consists of an ion source (the inductively-coupled plasma), and a mass analyzer (typically a quadrupole mass spectrometer), although systems employing high resolution (HR) double-focusing magnetic sector field mass analyzers are also employed. Typically, for total Se analysis the sample introduction system consists of a liquid handling autosampler, peristaltic pump, pneumatic nebulizer and spray chamber and injector.

High resolution-ICP-MS systems are capable of resolving some Se isotopes from polyatomic and isobaric interferences of similar mass (see Table 3), but fail to do so for the major Se isotopes $^{78}$Se and $^{80}$Se in a reliable manner in real-world samples. Quadrupole ICP-MS systems (by far the most common), do not have sufficient resolution to resolve polyatomic species from Se ions (nominally 1 atomic mass unit); therefore, great care must be exercised in order to evaluate, document and minimize potential biases created by these interferences. Solid samples must be dissolved before analysis, typically using microwave assisted acid digestion. There are methods for direct sampling of solid samples, such as laser ablation, but the obtained results are hard to quantify. Analysis of Se by ICP-MS should be performed by analysts who are well versed in the recognition and correction of spectral, chemical and physical interferences. The United States Environmental Protection Agency (US EPA) has published standardized methods for analysis of trace elements, including Se, by ICP-MS (EPA 6020a) (USEPA 1998). This method is the basis for Se determinations by ICP-MS for regulatory compliance in samples other than drinking water in the United States. For drinking water samples, the equivalent method is described by US EPA method 200.8 (USEPA 1994), but this method suffers from the shortcoming that it prescribes the use of the $^{82}$Se isotope for quantification, but does not allow the CRC technology to remove the potential interference on this mass by bromine, so that erroneous results can be obtained in bromide-containing waters.
Without a means of resolving or removing interferences, such as collision/reaction cells (CRCs), isotopes used for measurement with a quadrupole ICP-MS must be carefully selected. For example, the most abundant Se isotope, $^{80}$Se, cannot be used for trace level Se measurements without the use of a CRC, because of a severe interference from argon (Ar) dimers ($^{40}$Ar$^{40}$Ar) resulting in an unacceptably high background signal. Several abundant Se isotopes suffer from similar interference from Ar dimers as well as isobaric interferences from germanium (Ge) and krypton (Kr) isotopes (see Table 3). When chlorine (Cl) concentrations are low (<10 mg Se/L), $^{77}$Se provides relatively accurate measurements when an instrument with a reaction or collision cell is not available (Vanho et al. 1994).

Table 3. Isobaric and polyatomic interferences for naturally occurring Se isotopes with elemental correction equations for isobaric interferences (May and Wiedmeyer 1998).

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Mass</th>
<th>Abundance [%]</th>
<th>Corrections</th>
<th>Potential Interferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{74}$Se</td>
<td>73.9225</td>
<td>0.89</td>
<td>-1.317357 * $^{72}$Ge</td>
<td>Ge, ArS, Nd$^{2+}$, Sm$^{2+}$, Sm$^{3+}$</td>
</tr>
<tr>
<td>$^{76}$Se</td>
<td>75.9192</td>
<td>9.37</td>
<td>-0.276325 * $^{72}$Ge</td>
<td>Ge, ArS, Ar$_2$, Sm$^{2+}$, Eu$^{2+}$, Gd$^{2+}$</td>
</tr>
<tr>
<td>$^{77}$Se</td>
<td>76.9199</td>
<td>7.63</td>
<td></td>
<td>ArCl, Ar$_2$H, Sm$^{2+}$, Gd$^{2+}$</td>
</tr>
<tr>
<td>$^{78}$Se</td>
<td>77.9173</td>
<td>23.77</td>
<td>-0.030461 * $^{83}$Kr</td>
<td>Kr, Ar$_2$, Dy$^{2+}$, Gd$^{2+}$</td>
</tr>
<tr>
<td>$^{80}$Se</td>
<td>79.9165</td>
<td>49.61</td>
<td>-0.198433 * $^{83}$Kr</td>
<td>Kr, Ar$_2$, BrH, Dy$^{2+}$, Gd$^{2+}$, Dy$^{2+}$</td>
</tr>
<tr>
<td>$^{82}$Se</td>
<td>81.9167</td>
<td>8.73</td>
<td>-1.007833 * $^{83}$Kr</td>
<td>Kr, Ar$_2$H, BrH, SO$_3$, Dy$^{2+}$, Ho$^{2+}$, Er$^{2+}$</td>
</tr>
</tbody>
</table>

Aside from spectral interferences, ICP-MS (as well as ICP-OES) has two other major types of interferences, which are related to changes in the sample uptake rate in the nebulizer and to changes in the plasma properties. The former are caused by changes in sample viscosity (e.g. due to high salinity), and non-specifically depress the signals for all elements measured, so they can be compensated for by using internal standardization with any suitable element (e.g. rhodium). The latter affect only elements with high ionization potential like Se, and can result in either increased (e.g. by organic carbon) or decreased (e.g. by high sodium concentrations) ionization, so it can only be compensated for with either internal standards that mimic the ionization properties of Se closely (such as Te) or by isotope dilution.

ICP-MS with Collision/Reaction Cell (CRC) Technology

A number of devices have been introduced in the past decade that can be used to reduce or eliminate polyatomic interferences in ICP-MS. These devices allow for the determination of Se using $^{80}$Se and/or $^{78}$Se, the most abundant isotopes. This helps to decrease detection limits for Se and avoid polyatomic interferences created from sample constituents or Ar from the plasma. The most commonly used of these devices are the Dynamic Reaction Cell (DRC; PerkinElmer Sciex), the Octopole Reaction System (ORS; Agilent Technologies) and the collision cell (CC; Thermo Instruments). The DRC and ORS methods are based upon similar principles, but have a few key differences. The DRC system consists of a reaction cell containing a quadrupole placed in the path of the ion beam between the ion optics and the mass analyzer quadrupole. The cell can be pressurized with a variety of gases including NH$_3$, CO, CH$_4$ and dilute H$_2$. For Se, both CH$_4$ and CO are very effective at removing Ar interferences. Removal of interferences occurs through gas phase proton transfer or electron transfer reactions in the DRC. The quadrupole set in the DRC scans synchronously with the
mass analyzer quadrupole to reduce unwanted side reactions that could generate interferences by destabilizing intermediates. In the ORS system, an octopole is enclosed in a chamber which can be pressurized with gas, similarly to the DRC system; however, the octopole acts as a simple ion guide rather than a mass filter. Typically, the octopole reaction cell is pressurized with helium (He) or hydrogen (H₂). In addition to charge transfer and proton transfer reactions in H₂ mode, polyatomic interferences can also be eliminated by creating a kinetic energy barrier or through collisional dissociation in the ORS system when He is used as a cell gas (McCurdy and Woods 2004). Because polyatomic ions always have larger radii than their monoatomic counterparts, they participate in more collisions resulting in a greater reduction in their kinetic energy in the octopole than monoatomic ions. The bias of the octopole is tuned so that only monoatomic ions have sufficient kinetic energy to exit the octopole and enter the quadrupole mass analyzer. Use of either of these systems has been shown to be effective for Se analysis (Reyes et al., 2003; Hattendorf and Günther, 2003). The U.S. Environmental Protection Agency has taken the position that CRC technology may be used for the analysis of samples other than drinking water (EPA method 6020A) provided that all of the relevant data quality objectives (DQOs) are met; however, their use for drinking water analysis (i.e., samples for regulatory monitoring under the Clean Water Act, Safe Drinking Water Act and the National Pollution Discharge Elimination System; EPA method 200.8) has not been approved.

ICP-OES for Analysis of Selenium
Selenium may be analyzed by inductively-coupled plasma-optical emission spectrometry (ICP-OES; also known as inductively-coupled plasma-atomic emission spectrometry or ICP-AES). In ICP-OES, elements are quantified by measuring light emitted from excited ions and atoms at characteristic wavelengths rather than a mass spectrometer. Detection limits are relatively high (on the order of 10 or 100µg/L for axial and radial mode, respectively) in comparison to ICP-MS due to poor emission intensity for Se relative to other elements. These detection limits make it difficult to analyze Se in digested solid samples and nearly impossible to analyze Se in water samples. The detection wavelengths for Se are also in the deep UV region of the spectrum, which can sometimes hamper quantification. The primary detection wavelength (196.026nm) for Se also has a minor Fe interference, complicating analysis of samples with extremely high Fe and low Se concentrations. For these reasons, ICP-OES has not been commonly applied as a method of choice for Se analysis in environmental samples.

AAS and AFS for Analysis of Selenium
Atomic absorption spectrometry (AAS) has traditionally been the standard method for the determination of most trace elements. The measurement involves atomizing the sample (i.e., creating individual Se atoms in the gas phase in their electronic ground state) at elevated temperature, then irradiating these atoms with a beam of Se-specific radiation, and finally measuring the decrease of the initial radiation intensity due to absorption of this specific radiation by the Se atoms, which is proportional to the concentration of Se in the original sample via Lambert-Beer’s law. Boosted hollow cathode lamps or electrodeless discharge lamps provide the Se-specific radiation with high specificity and intensity. Traditionally, flames were used for atomization in AAS, but this is not very efficient for Se. Graphite
furnaces (GF) are more efficient, but are specific to the actual sample in their operation, and thus require a very experienced operator. However, they offer the additional advantage of being able to handle solid samples. Currently, AAS is usually used with hydride generation as a sample introduction/pretreatment step for Se analyses, because this yields extremely low detection limits in waters. Here, Se is volatilized as SeH₂ by reaction with borohydride under acidic conditions, and the resulting hydrogen stream can be used to create an H₂-microflame, which serves as an atomization source. The principle and problems of the HG technique are discussed in several sections below.

In the last decade, atomic fluorescence spectrometry (AFS) has become popular for Se determinations, because it yields even lower detection limits than AAS, and uses a much simpler (and cheaper) piece of instrumentation. The analytical principle is similar to AAS, only that the initially adsorbed Se-specific radiation is measured in the fluorescence mode, which conceptually involves waiting until the excited atom re-emits the absorbed quantum (which happens on the order of milliseconds) and measuring the appearance of this fluorescence intensity. Although AFS can be accomplished for many elements using an ICP for atomization, it is practically used only in combination with HG, and thus limited to hydride forming elements like Se. All other things being equal, HG-AFS is more sensitive than HG-AAS because it measures a small fluorescence signal relative to no background, whereas HG-AAS measures a small decrease of a large initial intensity by difference. In reality, though, the achieved instrumental sensitivity depends strongly on how well the HG step and the AFS or AAS instrument are optimized, so often comparable or better sensitivity has been reported for HG-AAS compared to HG-AFS.

**INAA for Analysis of Selenium**

Instrumental Neutron Activation Analysis (INAA) is an excellent method for total elemental analysis since sample manipulations such as digestion or dilution are not typically required before analysis. Furthermore, simultaneous elemental analysis of virtually the entire periodic table can be achieved without the usual concerns regarding differences in analytical recovery and sensitivity. Sample mass considerations are less of a problem with this method than in some of the other analytical approaches since very minute samples can still provide sufficient signal for accurate and precise quantitative analysis. However, because the analysis requires irradiation of samples and subsequent detection of the 162keV γ-rays from $^{77m}$Se, using this method requires collaboration with scientists at research reactors. The Se analysis method is described by McKown and Morris (1978), and it has been applied to a wide variety of inorganic and biological specimens. Small samples of tissue, usually 200-400mg, are placed in quartz cuvettes and briefly irradiated. Radioactivity in the sample is measured on the face of a germanium-lithium detector and the 162keV peak area is integrated. Peak areas correlate linearly with Se content up to 30nmol of Se, with a detection limit of approximately 0.5nmol. Contrary to all other analytical techniques discussed here, INAA yields a mass-based response, not a concentration-based response, so a direct comparison of instrumental detection limits can be performed only on samples with equal volumes or masses. Neutron activation analysis should be considered for benchmarking new methods and sample analysis procedures where potential for significant Se loss or contamination are concerns that cannot be otherwise addressed. Batch analysis by neutron activation analysis is not a limiting issue so entire experiments can be run by INAA.
Analysis of Selenium and its Species in Discrete Sample Types

Determination of Total Selenium in Waters

Since Se is not highly particle-reactive under typical environmental conditions, it is normally not necessary to incorporate the particulate Se fraction in the analysis of total Se concentrations in waters. Instead, the determination of total dissolved Se in a filtered sample (< 0.45µm or < 0.2µm) will normally yield approximately the same result. In waters with unusually high particle load (e.g., waters from industrial treatment processes involving precipitation reactions), though, this general practice may become problematic.

Consequently, a digestion (meaning a process that dissolves all particulate matter and releases the associated trace elements into solution) is typically not performed (or necessary) for the determination of total (dissolved) Se in waters. If a particle digestion is necessary (or performed), any suitable mixture of chemicals that accomplishes the complete digestion of the particulate material is acceptable for use, and heat and pressure can be used to accelerate the process (e.g., in a closed microwave bomb digestion); cf. section on digestion of soils, sediments and geological raw materials below. One has to distinguish this digestion process from other chemical pretreatment steps that may be necessitated by the specific analytical technique used for the measurement (see Table 4), if that analytical method requires all Se to be present in a specific chemical form (like HG-based methods require conversion of all Se to selenite prior to measurement). Unfortunately, such pretreatments are often also referred to as “digestion”, when they are technically derivatization techniques (because they involve conversion of one Se species into another).

The current US EPA water quality guidelines define their acute and chronic Se criteria as “total recoverable” concentrations, and acknowledge the low particle reactivity of Se in ambient waters by suggesting conversion factors of 0.996 and 0.922, respectively, for their acute and chronic fresh water Se criteria (USEPA, 2008). In our opinion, though, there are three fundamental problems associated with this approach, based on which we recommend that “total recoverable Se” measurements should not be performed, but substituted with “total dissolved Se” measurements. First, it cannot be determined directly (by comparing “total recoverable Se” and “total dissolved Se” concentrations in the same sample) what fraction of the total Se in a water sample is actually particle-bound, because the permitted analytical uncertainties for Se in official US EPA methods (e.g. ± 15% at the 100µg/L level in US EPA method 200.8) are larger than the assumed particulate Se fractions (0.4 or 7.8%; see above), so the validity of the above numbers for a specific situation cannot be tested. Second, although US EPA-approved analytical methods (e.g. method 200.8) provide detailed instructions regarding exactly how “total recoverable” trace element concentrations are to be determined, practice shows that many analytical labs deviate from these instructions. Since the obtained results depend very strongly on the exact “digestion” conditions used, caution should be used when interpreting results obtained by incomplete digestion/leaching of the particulate matter. Finally, “total recoverable” trace element concentrations have no substantial scientific meaning, because they do not represent any defined geochemically- or biologically-active fraction of the total element concentration.
Table 4. Techniques for total Se analysis in filtered or digested water samples

<table>
<thead>
<tr>
<th>Technique</th>
<th>Typical instrumental</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Special considerations and QC requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended techniques</strong></td>
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<tr>
<td>ICP-MS (CRC)</td>
<td>0.01 µg/L</td>
<td>fast; can quantify other elements simultaneously; less polyatomic spectral interferences than conventional ICP-MS</td>
<td>not widely available in commercial laboratories; not accepted by U.S.EPA for regulatory monitoring under the CWA or SDWA; requires expert operator</td>
<td>TSe results obtained via at least two different Se isotopes must agree within 10 %, and have to be lower than the results obtained via other Se isotopes</td>
</tr>
<tr>
<td>HG-AFS / HG-AAS</td>
<td>0.001 - 0.1 µg/L</td>
<td>widely available in commercial laboratories; no spectral interferences; simpler and cheaper than ICP-MS; accepted for regulatory purposes</td>
<td>single element technique; response is strongly species-dependent: chemical pretreatment required; interferences in HG step</td>
<td>requires complete conversion of all Se species in sample to Se(IV); conversion efficiency must be demonstrated for Se(VI) and SeMet by separate pre-conversion matrix spikes; recovery should be &gt; 80 % for Se(VI) and &gt; 90 % for SeMet</td>
</tr>
<tr>
<td><strong>Alternative techniques</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICPMS</td>
<td>0.1 µg/L</td>
<td>fast; can quantify other elements simultaneously; commonly available in contract laboratories; accepted for regulatory purposes</td>
<td>major Se isotopes cannot be measured; many polyatomic spectral interferences, especially in saline waters; requires experienced operator</td>
<td>TSe results obtained via at least two different Se isotopes must agree within 10 %, and have to be lower than the results obtained via other Se isotopes</td>
</tr>
<tr>
<td>GF-AAS</td>
<td>1 µg/L</td>
<td>very susceptible to matrix interferences; requires experienced operator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INAA</td>
<td>40 ng Se absolute; = 100 µg/L (for a 0.4 mL sample); concentration-based detection limit depends on sample volume</td>
<td>absolute measurement; reference method; no danger of sample contamination; requires no sample preparation; multi-element method</td>
<td>slow; expensive; requires access to nuclear research reactor</td>
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</tr>
</tbody>
</table>

Digestion procedures are generally not a significant source of Se, and the corresponding digestion blanks are often close to the instrumental detection limits. Likewise, sampling and storage artifacts are not a major concern for the determination of total Se in waters, as most commonly used sampling containers and equipment (such as tubing and filters) are “clean” with respect to Se without any pretreatment, and do also not adsorb significant amounts of Se. Acidification of waters with 0.1% (V/V) HNO₃ is a good way to prevent the precipitation of major element minerals during storage, which could lead to Se losses.
Due to the widespread availability of ICP-MS in commercial laboratories nowadays, the officially recommended methods for the determination of total Se in waters (e.g. US EPA methods 200.8 and 6020a) use this analytical technique. However, this approach has a number of pitfalls, which can be avoided only by experienced operators and may necessitate the use of advanced technology (like the CRC technology discussed previously). ICP-MS should generally achieve instrumental detection limits < 0.1µg/L for total Se in water, and this works reliably in fairly “clean” waters. However, waters with complex matrices often pose challenges with respect to spectral matrix interferences (as discussed previously).

Aside from the general QC criteria listed above, some specific data quality objectives apply to ICP-MS data (Table 4). In order for a total Se determination to be acceptable, at least two Se isotopes must be measured and must yield concentration results within 10% of one another; all other Se isotopes should yield higher results. The two main Se isotopes, $^{80}\text{Se}$ and $^{78}\text{Se}$, would be the ideal monitoring isotopes, because they yield the lowest detection limits and have very different interferences, but this can be accomplished reliably only with the reaction cell technology. The collision cell-based instruments yield good results for quantification via $^{78}\text{Se}$, but may require the use of the minor isotopes $^{77}\text{Se}$ or $^{76}\text{Se}$ for confirmation purposes, because the $^{40}\text{Ar}^+\text{Ar}^+$ background on $^{80}\text{Se}$ may be too high to accomplish low detection limits. Additionally, the isotopes $^{80}\text{Se}$ and $^{82}\text{Se}$ suffer from major HBr$^+$ interferences in waters that contain elevated bromide concentrations (which is the case for several types of important industrial process waters, as well as marine waters); this interference is typically not removed by the collision cell technology (so $^{77}\text{Se}$ or $^{76}\text{Se}$ must be used as the secondary isotope), but can be effectively eliminated by the reaction cell approach using NH$_3$ as a second reaction gas (besides the CH$_4$ used to eliminate the Ar$^+\text{Ar}^+$ interference on $^{80}\text{Se}$).

Simpler ICP-MS instruments that do not employ CRCs can not compensate for either of these interferences; contrary to the point of view propagated by the ICP-MS manufacturers, it is not recommended to address this problem with correction equations (included in the ICP-MS software), unless the analyzed samples are well characterized and very consistent between and within analytical batches. Consequently, conventional ICP-MS instruments (without CRCs) normally yield much higher detection limits, due to the combined effects of monitoring minor Se isotopes and having higher background signals. Unfortunately, the official US EPA approved methods explicitly preclude the use of CRC technology for the determination of Se by ICP-MS for method 200.8, resulting in erroneous results (often several orders of magnitude above the true concentration!) in certain types of waters, notably including bromide-rich industrial process waters.

While the CRC ICP-MS approach works well for most water samples, there are some limitations. As the total Se concentration decreases and the complexity of the waters increases, one reaches the point where the desired agreement between different Se isotopes can no longer be accomplished. To illustrate this: in a study on Se in waters in contact with fly ash at coal-fired power plants, quantification of total Se with different isotopes was reliable at concentrations above 1µg/L, but at lower concentrations, all five monitored Se isotopes typically yielded contrasting results. In this case, the best response is to pick the lowest result obtained for any Se isotope, because all spectral interferences are positive, but there is no guarantee that the lowest result is not still too high.
One possible solution to these interference problems is the use of hydride generation as a sample introduction technique. While this is typically done with AFS or AAS detection, there is no reason why it can’t be done with ICP-MS detection as well. The advantage of this approach is that most elements that cause spectral interferences in ICP-MS don’t volatilize during the HG process and are thereby removed prior to the Se measurement. This is one of the reasons why HG-AFS and HG-AAS have very few spectral matrix interferences. Additionally, one obtains lower detection limits due to the increased sample introduction efficiency of HG (theoretically 100%) compared to conventional nebulizer/spray chamber arrangements employed in ICP-MS measurements (< 5% sample introduction efficiency). The downside of HG as a sample introduction system is that it suffers from a number of different interferences, most of which are related to major matrix elements (particularly metals like Fe, Mn, Cu, Pb, Zn, etc.) reducing the efficiency of the HG process for Se. These effects need to be checked carefully by matrix spikes or the standard addition technique. The other major issue with HG as a sample introduction system (regardless of the detector used) is that all Se species present in the sample need to be converted quantitatively into selenite prior to the measurement, which can be complicated (see below) and needs to be checked rigorously for each sample type by separate blank spikes and matrix spikes with each of the major available Se species suspected to be present. To avoid these extra complications, ICP-MS (with CRC technology) is recommended as the default technique for the determination of total Se in waters under the caveats listed above. If this approach is unsuccessful, HG-AFS or HG-AAS can be useful alternatives for waters with high and variable spectral matrix interferences.

**Determination of Selenium Speciation in Waters**

Given that different Se species in waters behave differently with respect to bioavailability, persistence in ambient waters, and behavior during treatment procedures in industrial process streams, it is obviously important that reliable methods are employed for their analysis. In principle, two different approaches are commonly applied to the measurement of Se speciation in waters. The first is an operationally-defined fractionation approach based on the different HG behavior of individual Se species, whereas the second employs the on-line combination of a chromatographic separation and suitable atomic spectrometry detection for the direct determination of Se species. Before discussing the principal advantages and disadvantages of each, let’s start with a brief explanation of the principles underlying each of them and the fundamental differences between them.

The direct Se speciation method uses any suitable liquid-chromatographic (LC) separation mode to separate the different Se species, and then feeds them on-line into a suitable detector (ICP-MS is most convenient because it does not require any other sample treatment after separation). Because the predominant Se species in waters are anions, anion exchange chromatography (AEC) is a common and logical choice for an LC separation mode (see Table 5). Consequently, each Se species present in the water sample can be (at least theoretically) quantified directly independent of any other Se species and can be identified by its unique retention time, as long as it passes through the LC column and interacts with the stationary phase.
Table 5. Techniques for analysis of Se speciation in filtered or digested water samples

<table>
<thead>
<tr>
<th>Technique</th>
<th>Typical instrumental detection limit</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Special considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended technique</strong></td>
<td></td>
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</tr>
<tr>
<td>AEC-ICP-CRC-MS</td>
<td>0.01 µg/L</td>
<td>can detect and quantify unknown Se species; detection response is species-independent</td>
<td>sensitive to chromatographic artifacts, especially from acid in preserved samples; requires expert operator, labs may not be able to dedicate ICP-MS as chromatographic detector</td>
<td>TSe should be determined in the same sample to check Se speciation mass balance At least two Se isotopes should be monitored, and quantification via both should agree within 10 % for all detected Se species</td>
</tr>
<tr>
<td>AEC-HG-AFS / AEC HG-AAS</td>
<td>0.1 µg/L</td>
<td>can detect and quantify unknown Se species; detection response is species-independent</td>
<td>sensitive to chromatographic artifacts, especially from acid in preserved samples; requires expert operator, labs may not be able to dedicate ICP-MS as chromatographic detector; interferences may produce artifact peaks and complicate quantification</td>
<td>TSe should always be determined in the same sample to check Se speciation mass balance At least two Se isotopes should be monitored, and quantification via both should agree within 10 % for all detected Se species</td>
</tr>
<tr>
<td>HG-AFS / HG-AAS with selective sequential hydride generation</td>
<td>0.001 - 0.1 µg/L</td>
<td>works very well in marine waters</td>
<td>cannot detect unknown Se species; does not function in complex industrial process waters due to matrix interferences</td>
<td></td>
</tr>
</tbody>
</table>

**By contrast, the operationally-defined fractionation approach makes use of the fact that only selenite forms the volatile hydride SeH₂ by reaction with borohydride (BH₄⁻), and assumes that other Se species can be selectively converted into selenite by a sequence of chemical reactions under different conditions, so that each species can be separately measured using several sequential HG-AFS (or, less commonly, HG-AAS) determinations. A priori, this approach can identify and quantify only those Se species for which a defined set of chemical pretreatment conditions can be found that convert only this species quantitatively into selenite, while leaving all other Se species intact (or, at least, not converting them to**
selenite). Practically, such conditions have been described for only two Se species: selenate and “organic Se” (an umbrella term used to describe both defined organo-Se compounds as well as natural organic matter containing Se). For selenate, one uses a pre-reduction step that converts selenate to selenite, while for “organic Se”, in which Se is assumed to be present in oxidation states < +IV, an oxidation step is employed.

The vast majority of previous Se speciation measurements used a sequential selective hydride generation (SSHG) procedure popularized by Cutter (1978), in which selenite is measured directly in the sample, selenate is measured after pre-reduction in boiling HCl, and the remaining Se species are quantified after a complete oxidation with persulfate (S$_2$O$_8^{2-}$), followed by the same HCl pre-reduction (which is necessary because the oxidation may convert some or all of the other Se species to selenate). In the research conducted by Cutter, this fraction is correctly labeled as “reduced Se”, but unfortunately, many users of this SSHG speciation approach have used the term “organic Se” instead for the interpretation of their results, ignoring the possibility that reduced inorganic Se species could be present in certain waters. Normally, three separate measurements are performed on differently pretreated aliquots of the same sample (1. selenite alone without pretreatment, 2. selenite + selenate after reduction, and 3. “total Se” after oxidation + reduction), and then selenate and “reduced Se” are calculated by difference between these measurements (2. − 1. and 3. − 2., respectively). Lately, this approach has been modified by Chen et al. (2005) who use a UV-digestion to convert organic Se species into selenite, and can thus quantify it independently of selenate.

The best way to perform direct (sometimes also called “hyphenated”) Se speciation analyses is by LC-ICP-MS, because the ICP-MS detector yields a species-independent response. There are numerous publications that describe the use of HG-AFS or HG-AAS as Se-specific detectors for Se species separated by various forms of LC, but this has the fundamental disadvantages discussed for the HG technique in this manuscript, i.e. that some species are detected with lower sensitivity than others (or, in the worst case, not at all). The biggest advantage of LC-based Se speciation methods is that they have the potential to detect and quantify Se species that were not expected to be present in the sample, as long as those are separated from the known Se species. This is a big concern in water samples with unusual hydrochemistry (e.g., certain industrial process waters). Additionally, these Se speciation methods yield equal detection limits and reproducibility for all Se species. The shortcomings of LC-based Se speciation methods include the potential susceptibility of the chromatographic separation to matrix effects and the limited sample volume (maximum one mL) that can be analyzed, resulting (all other things being equal) in higher detection limits. On the other hand, other types of matrix effects, e.g., chemical or spectroscopic interferences in the detection process, are typically eliminated by the chromatographic process, because the interferent does not pass the LC column or elutes at a different time than the analytes.

The operationally-defined SSHG approach, by comparison, is limited to the determination of only those species that it was developed for. More importantly, it only works properly in water samples that have simple water chemistry, such as the marine waters for which it was developed. Unusual waters may contain large concentrations of matrix constituents that can interfere with the chemical reactions employed to fractionate the different Se species, and can thus lead to artifactual Se speciation patterns. This problem is specifically widely known
in the research community (although this is not documented well in the literature) for the selenate pre-reduction step, which is generally incomplete and strongly variable in complex water samples, leading to underestimated selenate results and also affecting the calculation of “reduced Se” by difference. The strong points of the SSHG approach include generally lower detection limits (due to larger sample volumes) and robustness towards high salinity and extreme pH.

As in any other speciation analysis, preservation of Se speciation in waters during sampling, transport and storage is a fundamental concern. However, for filtered oxic water samples containing only selenite and selenate, this does not represent a practical issue, as they are stable even without preservation for several weeks. Acidification with HCl (typically 1% V/V) is the most common method for stabilizing selenite and selenate in waters, and while it may not be necessary to prevent oxidation of selenite to selenate, it does prevent the precipitation of Fe and Mn minerals, which in turn could cause loss of selenite. Unfortunately, this preservation method influences the choice of analytical Se speciation method to some extent, as the acidic pH and high salinity of the preserved sample are generally less suitable for LC-based hyphenated Se speciation methods, but work well for the SSHG approach. For water samples containing any other Se species besides selenite and selenate, no systematic information exists regarding suitable preservation methods, and this may well depend on the chemical composition of each individual sample. In the absence of a “failsafe” procedure for stabilizing Se speciation in samples of unknown or unusual chemical composition, it is recommended that samples are not preserved chemically, stored cool and dark in the absence of an air headspace in the sampling container, and analyzed as quickly as possible after collection. As we learn more about the occurrence, identity and stability of Se species besides selenite and selenate in ambient and industrial waters, it becomes increasingly important to study the aspect of species preservation in a more systematic manner.

**Recommended Procedures and QC Measures for Water Analysis**

At this point there are no Se speciation methods officially approved and/or recommended by government agencies, so it’s up to the stakeholder and the involved analytical laboratory to identify the appropriate analytical method for the task at hand. It is important to gather all the QC information necessary to allow an independent evaluation of the generated data, should this become necessary. It is equally up to the stakeholder to define the requirements for the analytical performance criteria relative to the study goal, and it should be kept in mind that while many commercial laboratories may not be able to offer adequate Se speciation methods for some cases. Such analytical services are available from experienced commercial and academic groups, although they may be more expensive than less challenging conventional analyses.

If the hydrochemistry of the water samples to be analyzed suggests the possibility of other species besides selenite and selenate being present, particularly for anoxic ambient waters and any industrial process water, Se speciation analyses should be conducted by LC-ICP-MS, if available. Beyond the general QC requirements listed above, some specific QC considerations apply (cf. Table 5). Selenium in each species should be quantified using two separate Se isotopes, and the resulting concentrations should match (as a guideline: within
10% of each other if the concentration of a Se species is sufficiently above the detection limit. Only filtered samples can be analyzed (to prevent particles from interfering with the chromatographic analysis), and a proper Se speciation mass balance should be established; for this purpose, the sum of all determined Se species should be compared to an independently-determined total Se concentration measurement in the same filtered sample (see above for QC criteria).

Although the agreement between the sum of all Se species and the total dissolved Se concentration should fluctuate randomly around 100% ± the sum of all analytical errors (guideline: ±10%), experience shows that the sum of Se species is generally somewhat lower than the total dissolved Se concentration. A Se speciation mass balance of 90 ± 10% should be considered acceptable for a sample set, but larger systematic discrepancies indicate either some methodological problem or the presence of one or more Se species that do not pass the chromatographic column (e.g., colloidal Se species). If the sum of Se species is significantly higher than the total dissolved Se concentration, an unrecognized spectroscopic artifact in the LC detection is usually present or there is a fundamental issue with the method used for total dissolved Se determination.

Issues related to LC detection include chromatographic peaks that do not correspond to Se species (which can be identified by the signals on other monitored Se isotopes being absent or having the wrong intensity) and changes in Se detection sensitivity within one chromatographic analysis, which can be identified and compensated for by internal standardization (Te added continuously after the chromatographic separation) or by isotope dilution (a specific Se isotope added continuously after the chromatographic separation). Quantification of Se species eluting in the chromatographic dead volume should be avoided, as it usually yields erroneous results. If unknown Se species are encountered and quantified, it is imperative that it is demonstrated that the Se speciation method yields equal response for each known Se species available. If this is not the case, then each known Se species must be calibrated separately, and the known Se species eluting closest to an unknown Se species should be used for its quantification. Identification of Se species based on retention time alone should also be avoided, unless the Se speciation of the analyzed samples is well known. While ideally the identity of each Se species should be confirmed either by molecular mass spectrometry or using a second different chromatographic separation, this is not practical for routine analyses, so at the very least, matrix spikes should be used to test if the retention times of known Se species have shifted due to matrix effects.

A state-of-the-art LC-ICP-MS Se speciation method can achieve instrumental detection limits (in DI water as a matrix) of <0.01µg/L (Wallschläger and Roehl, 2001), but the method’s performance is typically limited by the samples’ matrix complexity. The practical method detection limit (= instrumental detection limit × dilution necessary to avoid chromatographic artifacts) needs to be at least ten times lower than the required Se concentration level of interest to make any meaningful quantitative Se speciation statements; for example, if Se speciation analyses are performed to evaluate treatment options to lower Se discharge concentrations below a water quality guideline of 1µg/L, then the method detection limit for each Se species needs to be ≤0.1µg/L.
No certified reference materials (CRMs) for Se speciation in waters are currently available, but this should not prevent the laboratory from analyzing a reference material that is certified for its total Se concentration. In practice, most CRMs, particularly the synthetic materials available e.g. from NIST, contain a stable mixture of selenite and selenate, and their sum usually adds up to the certified total Se concentration. Any Se speciation analysis report should be accompanied by documentation of method blanks (which are generally below the detection limit), CRM recovery (target 100 ± 10%), sample duplicates (target ≤ 10 % relative percent deviation (RPD)) and sample spike recoveries (target 100 ± 10 % for each available Se species). Occasionally, it is observed that Se species convert when they are spiked into a matrix; this is acceptable if the added Se is recovered in the form of another Se species, but problematic if the added Se “disappears”. If no ICP-MS is available for detection of Se species separated by LC, then HG-AFS or HG-AAS can be used alternatively. This will typically result in detection limits increasing by about one order of magnitude if all detectors are optimized properly, but is often still sufficient for the determination of Se speciation in either waters with elevated Se concentrations or in relatively simple oxic ambient waters. Since HG is employed as an on-line derivatization step here, only those Se species for which analytical standards exist can be quantified (due to the potentially different HG response of unknown Se species). It is also possible that some Se species present in the samples will not yield any HG response and might be overlooked; therefore, it is even more important with these detection principles that a proper mass balance assessment is performed using a technique that truly determines the total dissolved Se concentration (see above). All other QC requirements remain the same as for LC-ICP-MS. Instrumental detection limits around 0.1µg/L are achievable.

If possible, the original operationally-defined SSHG Se fractionation approach (Cutter, 1978) should be avoided, unless the user is certain that only selenite and selenate are present in their samples. Even then, it has to be checked carefully with blank spikes and matrix spikes how quantitative the selenate recovery is for each specific batch of samples. If the selenate recovery is < 80%, the calculated Se speciation results are probably not useful for quantitative purposes; specifically, one has to take this reduced recovery into account when interpreting the “reduced Se” concentrations. If the organic Se fraction is of special interest (probably only in relatively clean ambient waters), then the modified SSHG approach with UV-digestion (Chen et al., 2005) should be used. Detection limits < 0.01µg/L are achievable for both approaches. All other QC parameters remain the same as for the LC-based Se speciation methods, with the exception of worse reproducibility being acceptable for the selenate determination by difference (20% RPD should be the maximum variability, unless the selenate concentration is very low, and/or small comparable to the other Se species).
**Determination of Total Selenium in Geological Samples**

There is little to worry about with respect to sample collection and storage for Se analyses in soils/sediments, aside from homogeneity and representativity issues, and even less for geological raw materials. Blanks and losses are negligible for common sampling equipment, and it is usually sufficient to store soil/sediment samples cooled or frozen until analysis to suppress biological reactions that might induce Se loss via volatilization; geological raw materials don’t even require this precaution, with the exception of hydrocarbon mixtures that might volatilize and/or contain volatile Se compounds. The major issue associated with the determination of total Se in solids is the completeness of the matrix digestion process necessary to generate a liquid sample. INAA is generally used as the reference method for the determination of trace elements in solid samples, because it can measure solid samples directly, but there are some issues that make the INAA determination of Se challenging (see above). Nonetheless, any proper study of total Se concentrations in solid samples could benefit from an independent QC check of the digestion completeness by a solid state spectroscopy method (e.g., INAA), because it is difficult to achieve complete decomposition of the solid matrix.

If no solid state spectroscopic method is available to check the digestion completeness, Se recovery from suitable CRMs (i.e. those that match the nature of the studied soils, sediments or geological raw materials reasonably well with respect to Se concentration and general geological composition) and pre-digestion matrix spikes can be used alternatively to evaluate digestion completeness. Se recoveries > 90% can easily be achieved for both techniques, and anything less should not be accepted.

The main differences between rocks and ores on one side and soils and sediments on the other is that the former have a much larger grain size and are fairly homogenous in their chemical composition. Selenium in such materials is bound in the mineral lattice, and is therefore much less leachable than Se in soils or sediments. Consequently, the digestion of rocks and ores needs to be complete to release all Se prior to analysis, which requires much more drastic digestion conditions than for soils/sediments. To ensure that all rocks and ores are digested completely, regardless of their actual chemical composition, a procedure involving an open vessel hot plate digestion with a mixture of concentrated HCl, HNO₃, H₂SO₄, HClO₄, HF and H₂O₂ is employed, which decomposes nearly all known minerals quantitatively (Hageman et al., 2002). Coal can be digested by the same procedure, only that HF is not required for complete digestion. During this procedure, the digest is evaporated to near dryness, but it is important not to evaporate the liquid completely, as this will lead to Se volatilization. To address the problems associated with the large grain size of rocks and ores, they are crushed and ground prior to digestion.

In soils and sediments, Se is generally not incorporated into the lattice of mineral constituents, but is rather adsorbed on their surface. In particular, Se shows no great affinity for silicate minerals, so it is generally not necessary to incorporate HF into any digestion procedure. The main task is to oxidize Se⁰, sulfide minerals and organic matter quantitatively to release all Se bound in these fractions. This is accomplished by any strongly oxidizing acid mixture, such as HNO₃/H₂O₂ (at elevated temperature and pressure, if needed; e.g., by microwave digestion) or aqua regia. There is no need to employ perchloric acid; in fact, it should strictly be avoided for organic samples to avoid the risk of
explosions. Crude oils can be prepared for analysis in three different ways. The first is controlled combustion with oxygen (which can also be used for coal); this approach has the advantage that it uses no chemical reagents that could introduce Se contamination. Alternatively, the crude oils can be diluted with xylene; these diluted samples can then either be analyzed directly (e.g. by ICP-OES), or they can be mineralized with HNO₃ in a microwave digestion prior to analysis.

Table 6. Techniques for total Se analysis in geological materials

<table>
<thead>
<tr>
<th>Technique</th>
<th>Typical method detection limit</th>
<th>Advantages</th>
<th>Disadvantages (beyond table 4)</th>
<th>Special considerations (beyond table 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended techniques</strong></td>
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<tr>
<td>ICP-MS (CRC)</td>
<td>0.1 mg/kg (dw)</td>
<td>constituents of digested sample may create new interferences in CRC;</td>
<td>incompletely digested organic carbon can affect detection sensitivity for Se; needs to be checked with either standard addition or by using a suitable internal standard (e.g. Te), and corrected for, if necessary.</td>
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<td></td>
<td></td>
<td>concentrated acid matrix is incompatible with sample introduction and requires high dilution or matrix matching</td>
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<tr>
<td>HG-AFS / HG-AAS</td>
<td>0.1 mg/kg (dw)</td>
<td>less susceptible to acidity of digest than ICP-MS</td>
<td>HG procedure is very pH dependent; matrix matching between standards and samples is crucial</td>
<td>transition metals leached from the sample during digestion have strong matrix effects on HG procedure; matrix spikes are needed to check for HG efficiency (ideally for every single sample, unless bulk sample composition is constant within a sample batch).</td>
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<tr>
<td><strong>Alternative techniques</strong></td>
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<tr>
<td>ICP-MS</td>
<td>1 mg/kg (dw)</td>
<td>concentrated acid matrix is incompatible with sample introduction and requires high dilution or matrix matching; digestion acid mixture creates many spectral interferences; leached matrix constituents cause many spectral interferences</td>
<td>incompletely digested organic carbon can affect detection sensitivity for Se; needs to be checked with either standard addition or by using a suitable internal standard (e.g. Te), and corrected for, if necessary.</td>
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<tr>
<td>INAA</td>
<td>40 ng Se absolute; = 0.1 mg/kg (dw) (for a 400 mg sample); concentration-based detection limit depends on sample mass</td>
<td>eliminates problems associated with potential incomplete digestion for all other techniques</td>
<td>more potential spectroscopic interferences than for waters, because activated matrix elements are present at higher levels</td>
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<tr>
<td><strong>Unsuitable technique</strong></td>
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</tr>
<tr>
<td>GF-AAS</td>
<td>1 mg/kg (dw)</td>
<td>allows direct analysis of solid samples</td>
<td>GF procedure is extremely sample/matrix specific; requires a very experienced operator and very detailed knowledge of sample homogeneity and matrix composition</td>
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</tbody>
</table>
ICP-MS is recommended as the primary method of analysis for digests of raw geological materials, soils and sediments, but some of the digested materials described above pose particular challenges for ICP-MS determinations, which need to be considered carefully. Sediments and soils are generally unproblematic to analyze, once the digests have been diluted enough to overcome the issues associated with acidity and spectral interferences from the used acids (see below). It is important, though, that the organic matter in soils and sediments is mineralized (converted to inorganic carbon) completely during the digestion process; otherwise, the dissolved organic carbon remaining in solution can enhance the detection sensitivity for Se; this can be compensated for by standard addition, internal standardization with Te, or isotope dilution. The same problem occurs – in a more pronounced way – for incomplete coal and crude oil digests, and particularly for the xylene dilutions of crude oils. In addition, samples with high organic solvent concentration extinguish the plasma, so oxygen needs to be added on-line to sustain the plasma.

Rock digests contain high concentrations of matrix elements such as silicon (Si), aluminum (Al), Fe, S, calcium (Ca) and magnesium (Mg), which cause signal suppression in the sample introduction and ionization stages, which can be addressed by internal standardization. Additionally, they may cause specific spectral interferences on certain Se isotopes (e.g. $^{38}\text{Ar}^{40}\text{Ca}^+$ on $^{78}\text{Se}$) which need to be compensated for by using other Se isotopes for quantification, or eliminated by using the CRC technology. Likewise, the main constituents of ores may cause unusual spectral interferences in ICP-MS, so it is crucial to measure as many isotopes as possible, in order to check which ones are interfered with. In this regard, CRC may not always provide the solution to these problems, because it turns out that these major constituents may form new interferences in the CRC process. Experience shows that as long as the sample composition is well known and consistent, CRC can usually be used by an experienced operator to obtain reliable Se measurements, but if there is large heterogeneity between different samples analyzed together, then the number of arising interferences may be too large to be removed reliably by CRC; in such cases, double focusing (DF)-ICP-MS in the medium or high resolution mode may remove unknown interferences.

Since soil/sediment digests are very different in their composition than ambient waters, it is important (on top of the general QC measures described above and the specific considerations listed in Table 6) to employ matrix matching between calibration standards and samples to obtain accurate results, regardless of the analytical method used. Digests of geological raw materials, soils, or sediments have two properties that make them potentially challenging for the common Se determination methods: high acidity and strongly oxidizing conditions. The high acidity is particularly problematic for ICP-MS analyses, because the instrument sensitivity drops dramatically in such matrices. On the other hand, the residual chemical oxidants in the digests interfere with the principle of the HG procedure (which is a reduction process). Both effects can generally be overcome by dilution, because of the high Se concentrations in soil/sediment digests. However, aside from using matrix matching to eliminate these problems, one can also use post-digestion spikes to assess if the digests have been diluted sufficiently to overcome them.
Digestion blanks are generally much smaller than the Se concentrations in soils/sediments, but are typically significantly above the instrumental detection limits of common analytical methods, and need to be corrected for. It is not recommended to dry soil or sediment samples prior to digestion, due to the potential of Se volatilization, either by biotic processes at room temperature, or by chemical processes at elevated temperature. Consequently, the required dry weight determination should be performed on a separate aliquot of the soil/sediment sample, not on the one used for digestion. For a common soil/sediment digestion procedure (e.g. 0.1 g dry solids and 10mL acid), one can achieve detection limits around 0.01mg/kg (dw) with either ICP-MS or HG-AFS/AAS determination.

**Determination of Selenium Speciation in Geological Samples**

The fundamental challenge in determining Se speciation in solid samples is that the techniques generally used for liquid samples cannot be applied directly, with the exception of crude oils and related materials, for which methods commonly employed for organic substances, e.g. GC or LC separations, can be used in combination with ICP-MS detection. There are two possible solutions to this problem: one can switch to solid state spectroscopy techniques, or one can try to extract the Se species from solid samples and then analyze them by the same methods described for liquid samples. The fundamental problem of solid state spectroscopy methods, which have the potential to identify and quantify (Se) species directly in solid samples, is that they generally have very high detection limits, and are consequently generally unsuitable for the study of Se speciation in all but the most contaminated samples. In turn, the fundamental problems of extraction-based (Se) speciation approaches are that they may alter Se speciation during the extraction process, and that one never knows if an extracted Se fraction corresponds in its identity to the model compound for which the extraction procedure was developed. However, since liquid extracts of solid samples can be analyzed with low detection limits, these approaches allow the assessment of Se speciation in most studied solid samples.

In the future, solid state spectroscopy methods will hopefully be developed to the point where they completely replace extraction-based approaches for the determination of (Se) speciation in solids, but at this time, extraction-based procedures are still more useful for the analysis of Se speciation in solids with moderate Se concentrations. However, it is important that these fundamentally different approaches be used in a complementary manner in Se speciation studies so that the maximum amount of knowledge can be obtained. It needs to be recognized that the determination of Se speciation in soils, sediments and geological raw materials is not standard technique and generally not available commercially; if such information is required for a study, it is important to contract a research group that has specialized experience in performing this type of research project. The only major analytical concern related to sampling and storage is to maintain the oxidation state of the material until analysis. It is recommended that anoxic materials be either frozen or stored for a short period of time in a glove box, and handled (including all extraction steps) in a glove box prior to analysis. If the extraction-based approach is used, extractions should be performed as soon as possible after collection (within a month), and extracts should either be analyzed right afterwards, or preserved appropriately until analysis (see water section).
Table 7. Techniques for analysis of Se speciation analysis in geological samples

<table>
<thead>
<tr>
<th>Technique</th>
<th>Typical method detection limit</th>
<th>Advantages (beyond Table 5)</th>
<th>Disadvantages (beyond Table 5)</th>
<th>Special considerations (beyond Table 5)</th>
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<tbody>
<tr>
<td><strong>Recommended techniques</strong></td>
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<tr>
<td>AEC-ICP-CRC-MS 0.1 mg/kg (dw)</td>
<td></td>
<td>leached sample constituents (e.g. organic matter and iron) can create serious chromatographic interferences</td>
<td>leached organic carbon can affect detection sensitivity for Se; needs to be checked with a suitable internal standard (e.g. Te) introduced continuously after the chromatographic separation, and corrected for, if necessary</td>
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<tr>
<td>AEC-ICP-MS 0.1 mg/kg (dw)</td>
<td></td>
<td>leached sample constituents (e.g. organic matter and iron) can create serious chromatographic interferences</td>
<td>leached organic carbon can affect detection sensitivity for Se; needs to be checked with a suitable internal standard (e.g. Te) introduced continuously after the chromatographic separation, and corrected for, if necessary</td>
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</tr>
<tr>
<td><strong>Alternative technique</strong></td>
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</tr>
<tr>
<td>HG-AFS / HG-AAS 0.1 mg/kg (dw)</td>
<td></td>
<td>unknown Se species are often encountered in soil/sediment leachates, so HG-based detection methods often fail to yield accurate results for those Se species that are not undergoing chromatic separation</td>
<td>transition metals leached from the sample during digestion have strong matrix effects on HG procedure; matrix spikes are needed to check for HG efficiency (ideally for every single sample, unless bulk sample composition is constant within a sample batch)</td>
<td></td>
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<tr>
<td><strong>Unsuitable technique</strong></td>
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<tr>
<td>HG-AFS / HG-AAS with selective sequential hydride generation 0.1-0.01 mg/kg (dw)</td>
<td>has the potential to detect Se species that do not undergo chromatographic separation, e.g. Se bound to NOM or colloidal Se</td>
<td>leached matrix constituents cause very strong interferences in HG procedure</td>
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Of all solid state spectroscopic technique that may yield direct Se speciation information in solid samples, at this time X-ray absorption methods, such as extended X-ray absorption fine structure (EXAFS) and X-ray absorption near-edge spectroscopy (XANES), appear to be most promising in terms of eventually reaching detection limits that allow the analysis of most soils and sediments of interest. The big downside of these techniques is that they require the use of synchrotron based X-ray radiation sources, and are consequently available at only a handful of major research facilities all over the world, as well as requiring experts for analysis/interpretation of the generated data. Consequently, these techniques will never be routine analytical tools that can be used on large numbers of samples. At this time, the best X-ray beamlines can perform Se speciation analyses at concentration levels in the low mg Se/kg range (Andrahennadi et al., 2007), which is adequate for many, but not all soil, sediment and other geological samples of interest, so it remains to be seen when these techniques become routine.
instrumental capabilities reach the point where they can compete with the extraction-based approaches in terms of detection limits. However, these synchrotron-based methods are already very valuable for measuring Se speciation in rocks, ores and coals, simply because these materials are so insoluble that extraction-based procedures cannot be applied to them.

In order to assess Se speciation in soils or sediments using extraction-based approaches, one needs to employ a number of different extractions to release the individual Se species/fractions into solution, which in essence amounts to a sequential extraction procedure (SEP). However, it is inappropriate to use the conventional SEP scheme that was developed for studying cationic trace elements in soils and sediments, because Se forms very different species and has a very different chemistry compared to those elements. Instead, one needs to use a Se-specific SEP that focuses on the major Se fractions in the particular soil/sediment type studied. Inherently, there is no SEP that will work equally well for each type of soil/sediment (e.g. oxic vs. anoxic layers), because they differ strongly with respect to their overall composition and Se speciation, so one pretty much has to adopt an existing approach and modify it for the specific purpose. The general requirement is that the SEP will distinguish between all major Se fractions listed above, and that each is released quantitatively and selectively in one specific extraction step.

The procedures published by Chen et al. (2006), and Wright et al. (2003) provide useful starting points, but any modification thereof should begin with a mild extraction of the loosely-adsorbed Se species (e.g. with MgCl₂-solution). In this fraction, Se speciation can be determined, preferably by AEC-ICP-MS. The organic matter fraction is preferably extracted under alkaline conditions, rather than decomposed oxidatively, because it is hard to oxidize the NOM selectively without dissolving Se⁰ and sulfide minerals. Thereafter, Fe- and Mn-minerals can be dissolved reductively to release the strongly bound Se species. There is some debate on how to extract the Se⁰, but it appears that CS₂ (Chen et al., 2006) is a better suited extractant than sulfite (Velinsky and Cutter, 1990), because it doesn’t attack the sulfide mineral fraction. Finally, the sulfide mineral fraction can be dissolved by oxidation and, a mass balance check can be obtained by performing a complete digestion of any residual material, and comparison to the total Se concentration of the sample.

Beyond the general QC procedures outlined above and the specific comments in Table 7 for the analysis of soil/sediment extracts, little can be done to assess the accuracy of any SEP for Se in soils/sediments, due to lack of appropriate CRMs or even reference compounds that could be spiked to the sample prior to extraction and would properly represent the native Se binding states. In fact, it is common to see that Se species spiked to soils/sediments appear to change with increased reaction/equilibration time (“aging”), generally becoming less extractable. The only QC criterion that can be applied here is the total recovery of all spiked Se over the different SEP fractions. This is where comparison between SEP and XANES/EXAFS has great promise, because one could follow spectroscopically what has been extracted in each fraction if the solid state techniques were sensitive enough. While this doesn’t work on some ambient samples yet (due to detection limit problems), it can be used to troubleshoot and test SEPs with respect to the questions of selectiveness and completeness of individual extraction steps. It is crucial, however, that all SEP results be checked thoroughly for reproducibility by running matrix replicates, because small changes in the procedures can cause big changes in the results.
In conclusion, it is not advisable to interpret SEP results too much in a quantitative sense, particularly when an SEP is applied to different soil/sediment types in one study. Rather, SEP is a useful tool to establish relative differences between different soil/sediment samples, especially if the overall nature of the samples is relatively consistent within the sample set. Additionally, SEPs provide information that can be used to estimate bioavailability and environmental mobility of Se in a given studied ecosystem.

**Determination of Total Selenium in Biological Samples**

All methods used for analysis of Se in solid samples can be and are applied to analysis of Se in biological materials (see Table 8). As with solid geological samples, a good reference method for biological samples is INAA. X-ray fluorescence spectroscopy (XRF) is not applicable to most biological samples, because concentrations are typically well below the method detection limits. Due to the limited availability of INAA, biological samples are typically analyzed using other common detection methods such as AAS/APS or ICP-MS using certified reference materials to help verify the accuracy of the technique.

Analysis of biological samples using these techniques requires decomposition of the sample and extraction and/or dissolution of Se. Preparation of biological samples for analysis of total Se is typically accomplished by digestion of the samples in HNO₃, sometimes with the addition of H₂O₂, using a microwave digestion system. Samples are heated in sealed fluoropolymer digestion bombs to 180°C for at least ten minutes. Standardized methods for acid digestion of solid samples for analysis of trace elements (including Se) by ICP-MS follow EPA method 3052 (USEPA, 1996). Typically, samples with a mass < 0.5 g can be digested whole, while larger samples must be homogenized and subsampled. Dissected tissues or whole organisms should be collected using trace-element clean techniques. Acid washed plastic tools or other metal free materials are recommended. There is probably little risk of Se contamination from most metal implements; however, the use of stainless steel should be avoided if possible because Se is present in certain alloys.

Samples should be frozen or stored on ice immediately upon collection to avoid enzymatic conversion of Se to volatile species and stored frozen as soon as possible. Although there are advantages and disadvantages to drying samples, Se concentrations in biological samples are typically determined on a dry weight basis in order to minimize variation associated with moisture content of the samples. The contribution of volatile Se species in most tissue samples is small; however, it is important to consider the potential for volatization during the lyophilization process. Biological samples that contain significant fractions of volatile species (e.g., CH₃-Se-CH₃, etc.) are best analyzed fresh, with the percent moisture being determined from a separate aliquot. Materials which may contain significant fractions of volatile species include urine, plant, or microbial samples. Comparisons between lyophilized and non-lyophilized samples should be carried out if uncertainty exists.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Typical detection limit</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Special considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended techniques</strong></td>
<td></td>
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</tr>
<tr>
<td>ICP-MS (CRC)</td>
<td>0.005 mg/kg dry mass in solid samples.</td>
<td>Speed, sensitivity, ability to simultaneously quantify other elements. Reduction or elimination of polyatomic spectral interferences and reduction of method detection limits over conventional ICP-MS.</td>
<td>Some laboratories may not be equipped with CRC instruments. Not accepted by U.S.EPA for regulatory monitoring under the CWA or SDWA.</td>
<td>Requires acid digestion for solid samples and method of standard addition for high carbon content samples (e.g. tissues).</td>
</tr>
<tr>
<td>HG-AFS/HG-AAS</td>
<td>0.1 mg/kg (dw)</td>
<td>Widely available in commercial laboratories, no spectral interferences, simpler and cheaper than ICP-MS, accepted for regulatory purposes.</td>
<td>Single element technique, response is species dependent, chemical pretreatment required, interferences in HG step, not as sensitive as ICP-MS (CRC).</td>
<td>Samples must be in SeIV oxidation state before analysis. transition metals leached from the sample during digestion have strong matrix effects on HG procedure; matrix spikes are needed to check for HG efficiency (ideally for every single sample, unless bulk sample composition is constant within a sample batch)</td>
</tr>
<tr>
<td>INAA</td>
<td>40 ng Se absolute; = 0.1 mg/kg (dw) (for a 400 mg sample); concentration-based detection limit depends on sample mass</td>
<td>Eliminates problems associated with potential incomplete digestion for all other techniques</td>
<td>Limited availability of INAA facilities.</td>
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<tr>
<td><strong>Alternative techniques</strong></td>
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<tr>
<td>GF-AAS</td>
<td>1 mg/kg (dw)</td>
<td>Allows direct analysis of solid samples.</td>
<td>GF procedure is extremely sample/matrix specific; requires a very experienced operator and very detailed knowledge of sample homogeneity</td>
<td></td>
</tr>
<tr>
<td>ICPMS</td>
<td>0.1 mg/kg dry mass in solid samples.</td>
<td>Speed, sensitivity, ability to simultaneously quantify other elements. Commonly available at contract laboratories.</td>
<td>Matrix and spectral interferences. Some spectral interferences are difficult or impossible to correct. Requires operator experienced with recognition of Se interferences.</td>
<td>Requires acid digestion for solid samples and method of standard addition for high carbon content samples (e.g. tissues). Samples with high bromide or chloride content can suffer from polyatomic interferences.</td>
</tr>
</tbody>
</table>

Homogenization of larger samples can be accomplished by grinding the sample to a fine powder in liquid nitrogen using a ceramic mortar and pestle or by using an equivalent Se-free apparatus. When grinding and subsampling tissues or whole organisms, replicate subsamples should be digested in order to determine the heterogeneity of the subsample. Grinding to smaller particle sizes generally reduces the subsampling error.
Graphite furnace AAS, HG-AAS, and HG-AFS are commonly applied to analysis of Se in biological tissues. Specific considerations for analysis of biological samples are similar to the considerations for geological samples. Total decomposition of the sample, recovery of Se and sample representativeness are perhaps the chief concerns with these techniques. Method detection limits on the order of 0.1-1.0mg Se/kg can be routinely achieved.

The use of ICP-MS for analysis of Se in biological samples is widespread, due to the speed and sensitivity of the method and because concentrations of other elements may be determined simultaneously; however, extreme caution must be exercised due to the potential for interferences which can yield erroneously high concentrations. There are potential interferences, isobaric and polyatomic, for every isotope of Se. In standard ICP-MS of biological samples, ratios of isotopes that have a low enough background signal to be practically monitored seldom match the natural abundance. Careful selection of isotopes and/or use of a CRC can help to eliminate or reduce these interferences resulting in greater accuracy and reduced detection limits. In standard mode, monitoring of $^{77}$Se and $^{82}$Se is recommended, although often the two isotopes yield concentrations which do not agree. In this case, it is best to use the isotope yielding the lowest concentration since all spectral interferences cause positive bias. For this reason, it is essential to analyze certified reference materials that are as closely matched to the sample matrices as possible.

Commercial liquid Ar often contains Kr as an impurity which can complicate the use of $^{82}$Se, and $^{77}$Se suffers from an ArCl interference. In CRC mode, it is recommended to monitor $^{78}$Se and $^{80}$Se, which are the most abundant isotopes ($^{80}$Se may only be possible in instruments capable of using reaction mode). In either standard or CRC mode, Se is readily detected in the vast majority biological samples regardless of source. Method detection limits on the order of 0.005mg/kg dry mass are obtainable using $^{80}$Se in CRC mode. Method detection limits using $^{77}$Se and standard ICP-MS are typically on the order of 0.1mg Se/kg dry mass.

Selenium analysis in biological samples is complicated by the possibility of matrix interferences. If significant quantities of organic carbon are present in digestates as a result of incomplete decomposition of organic molecules, the intensity of the Se signal can be enhanced relative to standards, resulting in a positive bias in the measured concentrations (typically 10-20%, but as much as 100%; Larsen, 1998). Accurate determination of Se concentrations by ICP-MS using either standard or CRC mode often requires the use of either the method of standard additions or standard addition calibration (a variation of the method of standard additions) as a means of quantification (Figure 6). Alternatively, Te may be used as an internal standard or the isotope dilution method may be used if no Te is present in the samples. Isotope dilution quantification may be inaccurate if interferences on the enriched isotopic standard are present. The method of standard additions provides a good combination of simplicity and accuracy and is most suitable when there is a wide variation among samples in the composition of the sample matrix.
The disadvantage of this method is slow throughput because it typically requires 4-5 measurement actions per sample (see Figure 7). For each sample, Se and internal standard intensities must be recorded for a blank, unspiked sample and at least two levels of spiked sample. As an alternative, when sample matrices are consistent among samples (e.g., all samples are muscle tissue from the same fish species, etc.) the method of addition calibration may be used. In this case, the sample matrices must be nearly identical in terms of sample type, total dissolved solids and acid concentration. Most commercial ICP-MS instrument control and data analysis software packages are programmed to perform standard addition measurements. A description of both methods is provided in Figure 6.

**Figure 6.** Schematic of calibration methods for analysis of Se in digested biological samples by ICP-MS.

For the method of standard additions, the net intensities (after internal standard correction) of the unspiked sample and sample spiked with various concentrations of Se standard are plotted versus concentration. Linear regression is performed and the y-intercept gives the concentration in the unspiked sample ($A$; in this example, sample concentration = 2mg/L). For standard addition calibration, the unspiked matrix composite intensity is subtracted from each point, setting the y-intercept to zero. The slope is then used to calculate concentrations of all subsequent unknowns ($B$).
Figure 7. Determining sample concentration using the method of standard additions (line A) or standard addition calibration (line B).

It is extremely important to monitor several quality control parameters when analyzing Se in biological samples. Included in these are digestion replicates, standard reference materials, reagent blanks, post-digestion spike recovery samples, and post-digestion analytical duplicates. Limits of acceptability for these criteria are listed in Table 9 and are based upon U.S. EPA method 6020a (USEPA, 1998) for ICP-MS, but are also applicable to AAS and AFS. Post digestion spike recovery and different dilution replicates can be omitted when using standard addition calibration or standard additions, since these methods correct for matrix effects.

Table 9. Recommended quality control parameters for analysis of Se in biological samples. Each of the listed quality control parameters should be determined for every 20 samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Limits of acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
<td>generally below method detection limit</td>
</tr>
<tr>
<td>Post Digestion Spike *</td>
<td>75-110% recovery</td>
</tr>
<tr>
<td>Digestion Replicate</td>
<td>RPD &lt; 20%</td>
</tr>
<tr>
<td>Dilution Replicate (same and different dilution) *</td>
<td>RPD &lt; 10%</td>
</tr>
<tr>
<td>Certified Reference Material</td>
<td>75-125% recovery</td>
</tr>
</tbody>
</table>

It is essential that laboratory control samples (LCS) are analyzed along with unknowns, particularly when using ICP-MS, where spectral and matrix interferences are likely. A variety of biological certified reference materials (CRMs) are available which are certified for Se concentration. In North America, both the U.S. National Institute of Standards and
Technology (NIST) and National Research Council of Canada (NRC/CNRC) provide a variety of biological matrices certified for Se concentration. Included in these are lobster hepatopancreas (NRC-TORT-2), dogfish liver (NRC-DOLT-2), orchard leaves (NIST-1571), apple leaves (NIST-1515), Se enriched yeast (NRC-SELM-1), mussel tissue (NIST-2976). Uncertified Se values are provided for Lake Superior fish tissue (NIST-1946) and dogfish muscle (NRC-DORM-3). A certified concentration for Se in fish tissue is provided for Lake Michigan fish tissue (NIST-1947). In addition, the Commission of the European Communities, Community Bureau of Reference (BCR) has provided an informational or certified Se values for aquatic plant (BCR-60), olive leaves (BCR-62), hay powder (BCR-129), white clover (BCR-402), plankton (BCR-414), sea lettuce (BCR-279), skim milk powder (BCR-063R), bovine muscle (BCR-184), bovine liver (BCR-185R), pig kidney (BCR-186), whole meal flour (BCR-189), cod muscle (BCR-422) and mussel tissue (ERM-CE278).

**Determination of Selenium Speciation in Biological Samples**

A large variety of techniques have been employed to determine Se speciation in biological samples and there is no single technique that is applicable to every biological Se species. Selenium is extensively involved in biochemical pathways that are analogous to S. As a result, there are a large number of Se-containing metabolites in biological materials that make Se speciation analysis a complex task. It may be difficult or impossible to account for each Se species and establish mass balance in some samples. Analysis strategies should be designed to address specific objectives rather than to identify and quantify each Se species (See Table 10). There are no standardized techniques and there are few if any commercial laboratories that offer Se speciation analysis in biological samples as a routine service. At this point, determination of selenomethionine (SeMet) is the most well developed speciation analysis technique and SeMet is the only analyte for which a CRM exists (selenized yeast, SELM-1, National Research Council of Canada). Typically the techniques are employed by specialized research analytical laboratories using highly skilled analysts. The literature on biological Se speciation is vast and there are several excellent reviews of the literature (Lobinski and Szpunar, 1999; Szpunar and Lobinski, 2002; Uden, 2002; Polatajko et al., 2006).

Most modern approaches to biological Se speciation employ hyphenated analytical techniques combining a means of separation and a detection method, usually a form of mass spectrometry. The separation methods which have been most frequently employed include high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and gel electrophoresis (GE). Among the HPLC methods, reverse phase chromatography (RP), reverse phase ion pair chromatography (RPIP), ion exchange chromatography (IC) and size exclusion chromatography (SEC) are the most commonly employed (Polatajko et al. 2006). For small molecules, IC, GC, CE and RP are commonly employed, while IC, GE and SEC have been used for separating macromolecules such as proteins and small peptides. Gas chromatography is primarily used as a means of separating volatile Se species such as CH$_3$-Se-CH$_3$ and CH$_3$-Se-Se-CH$_3$ but it may also be used for separation of selenoamino acids after preparation of volatile derivatives. The chief advantages of GC are its high resolving power and sensitivity; however, tedious derivitization procedures must be used for ionic and non-volatile species.
These procedures also sometimes produce the same derivative for two or more compounds. For example CN derivitization forms CH$_3$SeCN from selenomethionine and Se-methylselenocysteine (Polatajko et al., 2006). Flow field flow fractionation (Fl-FFF) is also an area ripe for development as a means of separating Se-containing macromolecules, but it has seldom been exploited for Se studies.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC-ICP-MS</td>
<td>Sensitive, quantitative element specific detection of Se species.</td>
<td>Species identification can be somewhat circumstantial. Spectral interferences possible</td>
<td>Perhaps the best technique for quantification. Other techniques such as molecular MS can be used to confirm species identification.</td>
</tr>
<tr>
<td>IC-ICP-MS (CRC)</td>
<td>Sensitive, quantitative element specific detection of Se species. Spectral interferences reduced relative to conventional ICP-MS</td>
<td>Species identification can be somewhat circumstantial.</td>
<td>Perhaps the best technique for quantification. Other techniques such as molecular MS can be used to confirm species identification. Detection is more sensitive than without CRC technology.</td>
</tr>
<tr>
<td>CE-ICP-MS</td>
<td>High resolution separations, sensitive, element specific detection of Se species.</td>
<td>Species identification can be somewhat circumstantial.</td>
<td>Unlike IC, anions and cations can be simultaneously separated using CE. Typically a total consumption, direct injection nebulizer must be used which is prone to clogging.</td>
</tr>
<tr>
<td>RP-HPLC-ESI-MS</td>
<td>High resolution separations, definitive species identification.</td>
<td>Severe matrix interferences. Not highly quantitative, artifacts are likely.</td>
<td>Good technique for species identification; however, artifacts can be formed in the electrospray due to oxidation of Se to selenoxides. Difficult to use quantitatively.</td>
</tr>
</tbody>
</table>

Specific detection methods commonly employed include electrospray ionization mass spectrometry (ESI-MS) and ICP-MS (Chassaigne et al., 2000). While molecular MS methods such as ESI-MS can often provide unequivocal identification of Se-containing compounds, they have several disadvantages, oxidation of small Se molecules in the electrospray being chief among them. The advantage of ICP-MS as a detection system include its sensitivity and the ability to quickly and unequivocally identify and quantify Se containing fractions. While the ICP-MS is good at identifying which analytical fractions contain Se, it does not
offer the possibility of providing the molar mass of Se containing molecules or any information about their structure. Identification of specific compounds using ICP-MS relies on comparisons of the retention time between standards and unknowns. A robust approach to Se speciation in biological tissues employs a combination of ICP-MS and ESI-MS to provide the greatest breadth and depth of analytical information. For protein analysis in organisms for which protein or genomic sequence data are available, molecular MS also offers the possibility of protein identification. For macromolecular separations, techniques such as Fl-FFF or SEC are used to separate proteins based on their hydrodynamic radii. Multi-angle light scattering (MALS) and dynamic light scattering (DLS) in conjunction with a refractive index (RI) detector can be used to determine the molar mass (MALS and RI) or hydrodynamic radius (DLS) of proteins and other macromolecules without the need for estimation based on a standard curve.

Dissected tissues or other biological materials must be analyzed fresh or flash frozen in liquid nitrogen and stored at -80 ºC prior to analysis. Sample size requirements vary according to technique, sample type, and Se concentration, but generally, samples should have a mass of at least 100 mg fresh weight. Specific analytical methods and objectives determine the subsequent extraction methods for Se. Extraction methods should be carefully scrutinized for possible artifact formation. This can be accomplished using careful analytical spike recovery studies. For determination of selenoamino acid and inorganic Se species content, proteins must first be hydrolyzed using acid or enzyme mediated hydrolysis. Because of the redox-sensitive nature of Se, use of acid hydrolysis carries with it the risk of artifact creation. The use of methanesulfonic acid to catalyze hydrolysis under an inert atmosphere is perhaps the least likely to oxidize Se (Goenaga-Infante et al., 2008). Enzymatic hydrolysis is often used because it is milder than acid hydrolysis and not as likely to result in the interconversion of Se species. A broad spectrum protease such as pronase E or pronase K is an effective approach and can achieve quantitative recovery of total Se in soft tissue samples (Quijano et al., 2000). Both methods can effectively extract soluble Se species including the selenoaminoacids, selenate and selenite.

Analysis of selenomethionine (SeMet) and selenocysteine (SeCys), the most abundant selenoamino acids in plants and animals respectively, has been the subject of extensive research, particularly in terms of the characterization of dietary supplements and foodstuffs and animal feeds/forage (Stadlober et al., 2001). Selenomethionine has also been implicated as an important determinate of biological accumulation, food chain transfer, and ecotoxicity (Besser et al., 1993; Fan et al., 2002). Both ESI-MS and IC-ICP-MS are commonly employed for the determination of SeMet and SeCys. Selenate, selenite, SeMet, selenocystine, selenomethylcysteine, selenohomocysteine and others can be conveniently separated using anion exchange columns. Because selenocysteine (monomer) rapidly oxidizes to form selenocystine (dimer) through the creation of a diselenide bond, it is difficult to differentiate between the two forms and SeCys is quantified as selenocystine (Stadlober et al., 2001).
Table 11. Techniques for analysis of Se speciation in biological samples (macromolecules)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Information provided</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEC-ICP-MS</td>
<td>Distribution of Se (and other elements) in macromolecules as a function of molecular weight</td>
<td>High loading capacity, high sensitivity. Ability to examine co-occurrence with other elements</td>
<td>Relatively poor resolution.</td>
<td>Can be used to isolate Se containing proteins. Fractions can be collected for further characterization.</td>
</tr>
<tr>
<td>PAGE-LA-ICP-MS</td>
<td>Distribution of Se (and other elements) in macromolecules as a function of molecular weight</td>
<td>High resolution. Ability to examine co-occurrence with other elements</td>
<td>Low capacity, poor sensitivity.</td>
<td>Proteins must be highly enriched in Se to detect. Se which is not covalently bound may be dissociated from proteins.</td>
</tr>
<tr>
<td>PAGE-autoradiography</td>
<td>Distribution of Se (and other elements) in macromolecules as a function of molecular weight</td>
<td>High resolution, high sensitivity</td>
<td>Low capacity, requires dosing study organisms with radiolabled Se compounds.</td>
<td>Not applicable to study of organisms from nature. Requires use of radioisotopes. Highly sensitive.</td>
</tr>
<tr>
<td>MALDI-TOF-LC-ESI-MS</td>
<td>Sequencing and identification of Se containing proteins.</td>
<td>Maximum amount of information on protein identity Se substitution</td>
<td>Difficult with organisms for which protein sequence data not available.</td>
<td>Bioinformatics is the limiting factor for this technique.</td>
</tr>
</tbody>
</table>

Selenocysteine/selenocystine are poorly retained by ion exchange columns as zwitterions and may be difficult to separate from a number of cationic species such as the trimethylselenonium ion, selenopropionate and selenocystathionine. To separate these forms, cation exchange chromatography may be used (Larsen et al., 2001). In most biological samples, these metabolites are present in trace amounts; however, in plants that hyperaccumulate Se, they may be present at significant concentrations (Wu, 1998). Reverse phase, ion pair and cation exchange chromatography may yield better separation of some of the Se containing metabolites than anion exchange, but they do not separate selenate and selenite, which is important for environmental samples (Bird et al., 1997). Selenomethionine is well retained on most anion exchange columns due to hydrophobic interactions between the methyl group and the support matrix of the ion exchange resin. It tends to be well separated from other Se species, which aids in its identification and quantification. While CE and IC are the most common separations methods coupled to ICP-MS, CE, IC and RP HPLC are used with ESI-MS. The many complications in determining the selenoamino acid content of samples will likely prohibit routine analysis of these compounds by commercial laboratories; however, interlaboratory comparison studies have demonstrated the reproducibility of determining SeMet concentrations in selenized yeast tablets using a variety of techniques (Goenaga-Infante et al., 2008).

Extraction of Se-containing proteins for analysis is typically carried out by homogenizing tissues in a lysis buffer. The crude extract is then centrifuged to remove insoluble material and the membrane fraction and filtered prior to analysis. In order to pre-concentrate proteins a variety of precipitation methods may be employed; however, these methods may dissociate proteins from electrostatically bound forms of Se if they are present. While it has been most common to analyze the water soluble cell fraction which primarily contains the
cytoplasm, it is also possible to solubilize the membrane fraction using denaturants or detergents. Care must be taken to avoid proteolysis once the cells have been lysed. This can be accomplished by keeping the extract ice cold and analyzing immediately and/or using protease inhibitor cocktails. Separation of proteins can be accomplished by using SEC or FI-FFF on-line with ICP-MS as a detector. Peaks containing Se can be collected and further characterized with respect to molar mass and identity (Unrine et al., 2006). Another approach is to use gel electrophoresis to separate proteins on a mass basis. Detection of selenoproteins can be accomplished using laser ablation ICP-MS. Bands can be cut from the gel and eluted for further characterization.

Solid state speciation of biological tissues can be accomplished using XANES. However, collection of XANES spectra requires a high flux, tunable X-ray source, which means that it must be performed at a synchrotron radiation source as previously described. While Se concentrations in tissues are seldom high enough to perform bulk XANES, micron scale localized Se “hotspots” can be analyzed using microfocused X-ray beams. When performing micro-XANES, a thin section of tissue is mounted on a sample stage and translated in a raster pattern through the X-ray beam so that the atomic fluorescence from Se can be used to create a two dimensional map of Se. The elemental map is then used to identify regions of high Se concentration. These regions can then be further interrogated by collecting XANES spectra. The XANES spectra provide information on the oxidation state of the element in question (Se). Fitting the spectra of standards and unknowns using the method of least squares (linear combination analysis) can provide quantitative estimates of the oxidation state. Comparison of the shape of the XANES spectra with standards can also often provide clues as to the identity of the Se compounds such as coordination geometry. In some cases, where extremely high Se concentrations exist, EXAFS data can be collected providing information on the identity of elements bound to Se, bond lengths, and coordination geometry (Punshon et al., 2005). Typically, Se concentrations in localized areas must be in the tens of mg/kg for micro-XANES to hundreds of mg/kg for micro-EXAFS analysis.
Conclusions

Selenium's unique chemistry and biochemistry lead to challenges in analysis as well as in environmental biology, animal physiology and toxicology. The relatively small difference between deficiency and toxicity also make environmental studies of Se unusual. The healthy range of dietary Se concentrations is most common in the environment, but ecosystems with either deficient or toxic Se concentrations occur throughout the world. It is important to note that these regions would be devoid of animal life if they had not evolved finely tuned homeostatic mechanisms that can either conserve Se when it is poorly available in diet, or shed any excess Se body burden.

Aquatic ecosystems in regions with rich soil Se are vulnerable to toxicity because Se can be readily leached from soils. Fish and the invertebrates they feed on in aquatic ecosystems are less able to vacate Se-contaminated waters and have fewer homeostatic mechanisms to shed excess body burdens of Se. Selenium is deficient in the environment in far more locations than it is hazardously high, but the severity of the symptoms that accompany selenosis are more acute and therefore have been more readily recognized. However, it is also important to note that toxicity syndromes with similar symptoms but different etiologies have often been mistakenly attributed to selenosis.

Improved analytical capabilities have made it possible to distinguish selenosis from syndromes arising as a result of exposure to organic toxins, and further improvements in analytical capabilities will enhance understanding of selenosis. Previous studies have not examined the important influences of exposure to various Se species nor the generation of specific Se species as a consequence of high Se exposures. As a result, numerous questions remain about environmental effects and Se species-specific differences that influence sensitivities of various animal types to Se exposure. These questions will require more complete understanding of the molecular interplay between these Se species and the biomolecules they interact with in exposed animals.

The Se dose-response relationships have been characterized for induction of selenosis in various forms of animal life, but molecular mechanisms of selenosis remain undefined. Without understanding the etiological mechanisms of selenosis, determining environmental concentrations that result in dose-dependent toxic effects provides incomplete guidance to the regulatory agencies that must define indices to differentiate safe vs. harmful exposures. Since regulatory agencies are required to err on the side of caution, the lack of defined and physiologically meaningful analytical endpoints associated with adverse environmental outcomes is likely to result in regulatory controls that are more restrictive than necessary.

Identifying molecular species that vary in biological and toxicological importance requires being able to accurately and precisely measure them. Advances in analytical capabilities that enable determination of Se speciation will improve the abilities of environmental scientists to recognize how, and at what concentrations these molecular species induce toxicity. Eventually these capabilities will allow us to differentiate Se rich soils and waters that may be associated with beneficial effects from those that contain excesses that might cause harmful effects.
Recommendations

1. The biological and physiological requirements of adequate dietary Se intakes by wildlife and humans need to be considered by regulatory agencies in order to avoid mandating overly restrictive regulatory controls that inadvertently contribute to Se deficiencies.

2. The extents and limits of homeostatic responses for various life forms need to be better understood in order to prepare proper regulatory guidance regarding sources of Se release into the environment.

3. Adaptive tolerance to chronically high Se exposures appears likely to occur through inducible mechanisms of homeostatic control. These mechanisms need to be characterized.

4. To avoid systematic errors, data generated in support of Se risk assessment, management and remediation decisions need to employ the most accurate and precise analysis methods available.

5. Regulatory agencies should recognize the value of speciation-based information for Se studies in natural and industrial environments, and ask for this type of information to be generated and incorporated in studies when necessary.

6. Regulatory agencies should offer guidance on which methods for Se speciation analysis are adequate for such studies.

7. Total Se and Se speciation analysis should be used in a complementary and comparative manner for developing the best informed risk assessment, management and remediation strategies.

8. The toxicity of organic Se species such as selenomethionine must be differentiated from toxic effects of other organic forms that may accumulate as a result of selenosis.

9. The molecular mechanism of Se toxicity needs to be defined in order to identify measureable biochemical endpoints that distinguish Se exposure from selenosis.

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